PB91-137307

Methods for the Investigation and Prevention of Waterborne Disease Outbreaks

(U.S.) Health Effects Research Lab., Research Triangle Park, NC

Sep 90

U.S. Department of Commerce
National Technical Information Service
NTIS

Accepted date:

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before complet

HERL-0757

1. REPORT NO.
EPA/600/1-90/005a

2.

3. PB91-137307

4. TITLE AND SUBTITLE
Methods for the Investigation and
Prevention of Waterborne Disease Outbreaks

5. REPORT DATE
September 1990

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Gunther F. Craun

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NA AND ADDRESS
HERL/ORD/USEPA, Cincinnati, OH 45268

10. PROGRAM ELEMENT NO.
ABMC1A

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Health Effects Research Laboratory (HERL)
Office of Research and Development (ORD)
U.S. Environmental Protection Agency (USEPA)
Research Triangle Park, North Carolina 27711

13. TYPE OF REPORT AND PERIOD COVERED
Published Paper

14. SPONSORING AGENCY CODE
EPA-600/11

15. SUPPLEMENTARY NOTES
P.O. Gunther F. Craun, PSS/EB/HSD/HERL/USEPA (CI)

16. ABSTRACT

This work consists of two volumes. Volume I ~~is~~ a series of articles based on selected presentations made at the U.S. Environmental Protection Agency (EPA) and Association of State Drinking Water Administrators Workshop on Methods for Investigation of Waterborne Disease Outbreaks held on October 11-13, 1988, in Denver, Colorado. Articles were selected to provide background on the etiologies and causes of previous outbreaks, various aspects of epidemiologic methods, disease surveillance and reporting, and laboratory analysis. This is intended to serve as a handbook to assist in the investigation of waterborne outbreaks. A number of previously published articles have been reprinted in Volume II to supplement information contained in the handbook. These articles provide examples of outbreak investigations and surveillance activities. They have been reprinted to illustrate principles discussed in the handbook and compiled as a convenience to those investigators with limited access to library facilities. The contributions of the authors of both handbook and journal articles are gratefully acknowledged. Many of the authors of articles in the handbook provided substantial peer review assistance. In addition, Dr. Dennis Juranek, Centers for Disease Control, Atlanta, Georgia; Mr. Peter Karalekas, Jr., Water Supply Section, EPA, Boston, Massachusetts; and Dr. Vincent P. Olivieri, Johns Hopkins School of Hygiene, Baltimore, Maryland, served as peer reviewers for the handbook.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT
RELEASE TO PUBLIC

19. SECURITY CLASS *(This Report)*
UNCLASSIFIED

20. SECURITY CLASS *(This page)*
UNCLASSIFIED

21. NO. OF PAGES
328

22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

PB91-137307

United States
Environmental Protection
Agency

Office of Research
and Development
Washington, DC 20460

EPA/600/1-90/005a

September 1990

EPA

Methods for the Investigation and Prevention of Waterborne Disease Outbreaks

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA 22161

EPA/600/1-90/005a
September 1990

Methods for the Investigation and Prevention of Waterborne Disease Outbreaks

Edited by:
Gunther F. Craun

Health Effects Research Laboratory
U.S. Environmental Protection Agency
Cincinnati, OH 45268

i.a

Notice

The opinions presented in this document are those of the authors and do not necessarily reflect the views of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Preface

This volume is dedicated to the engineers, sanitarians, microbiologists, epidemiologists, and other health officials who have labored throughout this century to improve the quality of our drinking water. Because of their endeavors, we in the United States and most developed countries now enjoy a quality of life in which waterborne disease outbreaks are no longer a constant threat or danger. This status has been achieved by the tireless efforts of many and often with little public recognition.

Two individuals with whom I had the privilege to work, Abel Wolman and Leland McCabe, deserve special acknowledgment. Both devoted their careers to public health, and their contributions in this area have been noteworthy. My association with these individuals over the past twenty years seems all too brief, but I shall always remember them and their commitment to ensuring the safety of drinking water. I thank them for providing inspiration to me and others to continue our careers in this field.

Although this volume is testimony to the successful efforts which have reduced the incidence of waterborne disease in the United States, we must not forget that infectious diseases still remain a threat for billions of people in the developing world where water supplies are not as well protected from sewage and may be inadequately treated. The lessons we have learned are important for reducing the worldwide incidence of waterborne diseases. We must also continue our vigilance because a residual number of outbreaks still occur in the United States, indicating the vulnerability of our water supplies.

The ease with which people can travel from country to country and the general mobility of world populations have provided additional opportunities for the waterborne transmission of many infectious diseases. Pathogens may be carried by refugees and immigrants settling in the United States and by returning U.S. citizens who have traveled throughout the world. Whether these pathogens become important sources of waterborne infection depends solely upon the effectiveness of the natural barriers which protect water sources from sewage discharges and multiple water treatment barriers which remove and inactivate these pathogens.

We have learned that many of our water sources are inadequately protected and treated to prevent the transmission of giardiasis. The waterborne transmission of this disease was first reported in the United States in 1965 and has increasingly been reported since 1971. *Giardia* is now the most commonly identified pathogen in waterborne outbreaks. Contaminated surface water is a significant source of infection for giardiasis, and almost half of the outbreaks of waterborne giardiasis have occurred in surface water systems where the only treatment was disinfection. In addition to *Giardia*, a number of new etiologic agents have been recognized in recent years and as laboratory analysis and investigation procedures improve, more agents are likely to be identified. Some of the newly recognized pathogens include *Cryptosporidium*, *Campylobacter*, *Yersinia*, and Norwalk agent. Also described is a chronic diarrhea for which an agent has yet to be identified despite extensive laboratory analyses. In a recently investigated waterborne outbreak in Missouri during December 1989-January 1990, hemorrhagic *E. coli* serotype 0157:H7 was identified as the etiologic agent.

Only a fraction of the waterborne outbreaks that occur in the United States are recognized, investigated, and reported, and in only half of the reported waterborne outbreaks was an etiologic agent identified. We must improve the investigation and surveillance of waterborne disease to identify the causes of these outbreaks and the etiologic agents. Are outbreaks of acute gastroenteritis of undefined etiology caused by unrecognized viral, protozoan, or bacterial agents or by pathogens that could not be determined only because of delayed investigation or inadequate laboratory analyses? It is necessary to obtain more information on the pathogens causing these outbreaks in order to evaluate the effectiveness of drinking water regulations, surveillance activities, water treatment technologies and source water protection policies. The identification of *Giardia* as an important waterborne pathogen stimulated the research that has identified the types of filtration, operating parameters, and disinfection levels necessary for removal and inactivation of this pathogen. Water treatment research is now being conducted to obtain similar information for *Cryptosporidium*.

Regulations have been found insufficient to prevent the waterborne transmission of infectious disease, as outbreaks have occurred in systems that have not exceeded standards for coliforms and turbidity. Data from outbreaks clearly show that disinfection has not been effective as the only treatment for surface water sources; filtration, including any required pretreatment, may be required in all but exceptional situations to ensure the removal and inactivation of waterborne pathogens, such as *Giardia* and *Cryptosporidium*. Criteria have now been proposed for the filtration and disinfection of surface water supplies, and regulations are being revised for coliforms. Outbreaks have also occurred in filtered water systems where facilities have been improperly designed or have been operated in a poor or simply casual manner, emphasizing the need to pay greater attention to providing and maintaining effective, continuous water treatment.

All of the waterborne pathogens are transmitted through the fecal-oral route of exposure, and human sewage is an important source of water contamination. A review of the important waterborne diseases since the turn of the century indicates that new strategies are needed to prevent waterborne outbreaks. The important waterborne diseases in the earlier part of this century were typhoid fever and cholera. Although filtration played an important role in reducing the waterborne transmission of these diseases in areas where water sources were heavily contaminated, certain characteristics of these pathogens allowed development of water sources with minimal treatment. These diseases are transmitted exclusively among humans, and waterborne transmission could be prevented by protecting sources from sewage contamination. Since these pathogens are quite susceptible to disinfection, chlorination was found to be adequate treatment where water sources could be protected from discharges of human sewage. The more recently identified waterborne diseases such as giardiasis and cryptospordiosis are caused by protozoa which are much more resistant to disinfection. These protozoa can also cause infection at a much lower dose than *S. typhi* and *V. cholera*. In addition to being transmitted through human sewage, it has been found that wild and domestic animals are important primary or intermediate sources of infection for these protozoa and for other newly recognized pathogens as well. Since it is impossible to exclude animals from watersheds, greater emphasis must be placed on water treatment barriers. Although the protection of raw water quality is still important, it can only be considered one barrier to the transmission of waterborne disease, especially with increasing evidence of the importance of animals in the transmission of waterborne disease. The increasing potential for contamination of surface water sources warrants the maintenance of additional barriers – filtration and disinfection – to ensure adequate margins of safety.

Public health officials are committed to the multiple barrier concept which provides for sewage treatment, protection of water sources, and multiple water treatment processes. The validity of this concept has evolved from water treatment experiences and if properly applied can significantly reduce the risk of waterborne transmission of infectious disease. Outbreak data, however, indicate that more emphasis must be placed on the design and operation of filtration facilities if they are to be effective as part of the multiple barrier concept. Many years ago I recommended the disinfection of untreated ground water to reduce the occurrence of waterborne outbreaks. I am still of the opinion that this recommendation is appropriate, particularly for small systems where wells or springs may be subject to intermittent contamination that cannot be readily determined or prevented. This does not suggest a decreased emphasis on protection of ground-water sources from contamination and proper construction techniques for developing these sources, but instead would provide an additional barrier for an increased level of protection.

Almost half of the reported outbreaks were caused by contaminated, untreated, or inadequately treated ground water and one fourth were caused by untreated or inadequately treated surface water. Adequate, continuous disinfection of drinking water must continue as the final barrier against waterborne disease, but the question remains whether chlorine will continue to be the primary disinfectant. The concerns over the human health effects that may be associated with long-term exposures to chlorine or chlorinated by-products may require changes in disinfection techniques or the use of disinfectants other than chlorine. These concerns must be tempered with considerations of the benefits provided by chlorination. As Dr. Wolman has so aptly stated many times, the chlorination of water supplies has saved numerous lives. Reducing the levels of chlorine added to water or using alternate disinfectants may be appropriate to reduce or prevent the formation of by-products, but the effects of these actions on the occurrence of waterborne disease must be carefully weighed. Additional research is required. Little is known about potential by-products and adverse effects of the other disinfectants, and unresolved questions remain about the significance of the relatively low cancer risks reported from the epidemiologic studies of chlorinated water systems. Although the risks of chlorination are not as well recognized as the benefits, the prudent public health practice at this time should be to minimize exposure to chlorine and chlorinated by-products without significantly increasing infectious disease risks. Chlorination may be the final barrier against transmission of waterborne pathogens, but it must not be the only barrier. Properly designed and operated filtration plants can make disinfection more effective by removing turbidity and some substances that exert chlorine demand and by reducing microbiological contamination. When disinfection is only one part of the multiple barrier approach and not relied upon so heavily, lower concentrations of chlorine can be used, thereby lowering the levels of chlorinated by-products produced.

G.F.C.

Contents

Preface iii
Acknowledgments ix

I. Introduction

Review of the Causes of Waterborne Disease 1
The Safe Drinking Water Act and the Regulation of Microorganisms in Drinking Water 23

II. Disease Surveillance

Waterborne Disease Outbreak Surveillance: Federal Requirements and Responsibilities 29
Surveillance for Waterborne Illness and Disease Reporting: State and Local Responsibilities 39

III. Investigation of Waterborne Disease Outbreaks

Differences between Outbreak Investigation and Research Epidemiology 45
Epidemiologic Principles for the Study of Waterborne Outbreaks 55
Data Analysis: Estimating Risk 65
Avoiding Bias: Systematic and Random Error 75
Predicting Exposure to Water Contaminants in Distribution Systems 83
Treatment Plant Evaluation During a Waterborne Outbreak 127

IV. Engineering and Water Quality Concerns

Surface Water Source Protection 157
Principles of Water Filtration 169
Principles of Drinking Water Disinfection for Pathogen Control 185
Determination of C·T Values 193
Distribution Systems: Treated Water Quality Versus Coliform Noncompliance Problems 207

V. Sampling and Analytical Methods

Environmental Sampling for Waterborne Pathogens: Overview of Methods, Application Limitations, and Data Interpretation 223
Bacteriologic Analysis of Clinical Specimens in Waterborne Disease Outbreaks 235
Clinical Laboratory Diagnosis of Enteric Viral Diseases .. 243
Clinical Diagnosis of Enteric Protozoans 247
Analysis of Water Samples for Bacterial Pathogens 249
Virological Analysis of Environmental Water Samples ... 275
Field Method for Concentrating Viruses from Water Samples 285
Analysis of Water Samples for Protozoans 297

Epilogue 317

Acknowledgments

This volume is a series of articles based on selected presentations made at the U.S. Environmental Protection Agency (EPA) and Association of State Drinking Water Administrators Workshop on Methods for Investigation of Waterborne Disease Outbreaks held on October 11-13, 1988, in Denver, Colorado. Articles were selected to provide background on the etiologies and causes of previous outbreaks, various aspects of epidemiologic methods, disease surveillance and reporting, and laboratory analysis. This is intended to serve as a handbook to assist in the investigation and prevention of waterborne outbreaks. A number of previously published articles have been reprinted in a separate volume to supplement information contained in the handbook. These reprints provide examples of outbreak investigations and surveillance activities and illustrate principles discussed in the handbook. The contributions of the authors of both handbook and journal articles are gratefully acknowledged.

Many of the authors of articles in the handbook provided substantial peer review assistance. In addition, Dr. Dennis Juranek, Centers for Disease Control, Atlanta, Georgia; Mr. Peter Karalekas, Jr., Water Supply Section, EPA, Boston, Massachusetts; and Dr. Vincent P. Olivieri, Johns Hopkins School of Hygiene, Baltimore, Maryland, served as peer reviewers for the handbook.

Gunther Craun (EPA Health Effects Research Laboratory, Cincinnati, Ohio) selected the subject matter, edited and compiled the manuscripts, and provided substantive guidance for preparation of the articles. Susan Richmond of Eastern Research Group, Inc., Arlington, Massachusetts, provided editorial assistance. Carol Legg and Steve Wilson (EPA Center for Research Information, Cincinnati, Ohio) provided production assistance and figure preparation.

This report has been reviewed by the U.S. Environmental Protection Agency and approved for publication.

I. Introduction

Review of the Causes of Waterborne Outbreaks

by: Gunther F. Craun
Coordinator, Environmental Epidemiology Program
U.S. Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, Ohio 45268
(513) 569-7422

Introduction

On a national basis, the reporting of waterborne disease outbreaks is voluntary. Information on waterborne outbreaks is obtained from the scientific and medical literature and through the assistance of State and local health officials, epidemiologists, and engineers. The U.S. Environmental Protection Agency (EPA), Cincinnati, Ohio, and the Centers for Disease Control (CDC), Atlanta, Georgia, have cooperated in the reporting of waterborne outbreaks since 1971 and have made this information available annually (1-14). Reviews of the causes of waterborne outbreaks have been published for various time periods (15). This analysis updates previous information and includes outbreaks reported through 1985.

Reported waterborne outbreaks are those in which drinking water was implicated epidemiologically as the vehicle of illness transmission. In most of the outbreaks, water was found to be bacteriologically or chemically contaminated, but in only a few outbreaks was the etiologic agent isolated from drinking water. To be considered an outbreak, at least two cases of a disease must be reported so that a common source can be noted and investigated. Except in unique circumstances, such as a case of chemical poisoning in which the chemical is identified, a single case of illness cannot be recognized as having been caused by drinking water Single cases of infantile methemoglobinemia associated with high nitrate concentrations in water have been included in this reporting system since 1979.

Outbreaks are primarily associated with water used or intended for drinking or domestic purposes, but outbreaks associated with ingestion of contaminated lakes, springs, creeks, and other sources of nonpotable water are also included. Excluded from this analysis are waterborne outbreaks occurring in cruise ships operating from U.S.

ports and outbreaks among swimmers, bathers, and hot-tub users where ingestion of contaminated water was not the route of exposure.

For analysis, water systems are classified as community or noncommunity according to definitions contained in the Safe Drinking Water Act (SDWA). Two additional classifications, individual and recreational water systems, are not covered by SDWA. Individual water systems are used by residents in areas without community systems or persons traveling outside of populated areas who may consume water from nonpotable sources (e.g., backpackers, campers). Water systems involved in outbreaks where contaminated water was ingested while swimming, diving, bathing, etc., are classified as recreational.

I recently reviewed individual reports of waterborne outbreaks reported during 1971-1985 to ensure the accuracy of information on this subject. This review resulted in minor changes to data previously reported (1-13), including changes to dates, water system classifications, and deficiencies responsible for outbreaks. Because of reporting delays, some outbreaks had been identified previously by date of report rather than date of occurrence. Changes in water system classifications were required so that definitions would correspond to those in the Safe Drinking Water Act. Additional information obtained on water system deficiencies also resulted in some changes. These revisions were reported to CDC and have recently been published (14,16). Because these data are maintained and shared by CDC and EPA for periodic evaluations, it is important that the reported information be complete and accurate.

During the period 1971-1985, 502 waterborne outbreaks and 111,228 cases of illness were reported to CDC and EPA.[1] Although more outbreaks were reported during 1971-1985 than any previous 15-year period since 1920 (Figure 1.1.1), the number of reported waterborne outbreaks has declined since 1981. The number of cases reported during the most recent 15-year period is exceeded only by the number reported during the time periods 1936-1950 and 1920-1935 (Figure 1.1.2). The average number of cases per waterborne outbreak has fluctuated from a high of 572 during 1926-1930 and 681 during 1961-1965 to less than 200 cases per outbreak during six time periods, including 1981-1985. The incidence of waterborne disease in the U.S. population has also declined from approximately 8 cases of illness per 100,000 person-years during 1920-1940 to 4 cases per 100,000 person-years during 1971-1985 (15). With a few notable exceptions, reported waterborne outbreaks now tend to occur in small water systems, affecting fewer people. The number of deaths associated with waterborne outbreaks has decreased dramatically since 1920 (Table 1.1.1), and no deaths were reported during 1981-1985. Most previous deaths were due to typhoid; however, the primary causes of death in recent waterborne outbreaks have been shigellosis and chemical poisoning.

---

[1] The 502 outbreaks and 111,228 cases in this analysis include the 485 outbreaks (110,359 cases) reported in Reference 14 (also used by Tauxe in a subsequent article in this volume) and 17 outbreaks (869 cases) related to ingestion of contaminated water while swimming or other recreational activity. All of these outbreaks were reported in previous CDC publications (1-14).

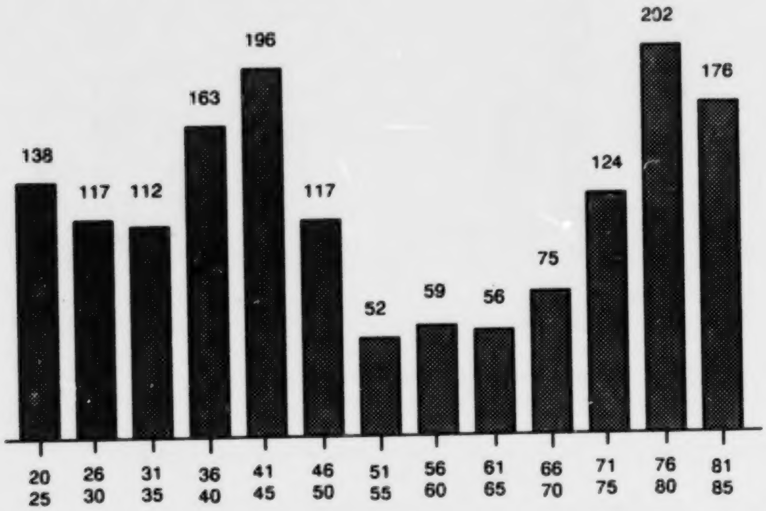


Figure 1.1.1. Number of waterborne disease outbreaks, 1920-1985.

During 1920-1935 waterborne outbreaks were more frequently reported in community systems, whereas in most succeeding years, outbreaks were more frequently reported in noncommunity systems (Figures 1.1.3 and 1.1.4). However, during the most recent 5-year period, more outbreaks were reported in community systems than in noncommunity systems. During 1971-1985, 42 percent of all reported waterborne outbreaks and 68 percent of the cases of illness occurred in community water systems; 43 percent of the outbreaks and 31 percent of the cases occurred in noncommunity systems (Table 1.1.2).

It is difficult to determine whether the decline in the reported number of waterborne outbreaks during 1981-1985 is due to the occurrence of fewer outbreaks or less active surveillance and reporting (Tauxe discusses this further in the article, "Waterborne Disease Outbreaks Surveillance: Federal Requirements and Responsibilities," on p. 29). The most dramatic decrease occurred during 1984 and 1985

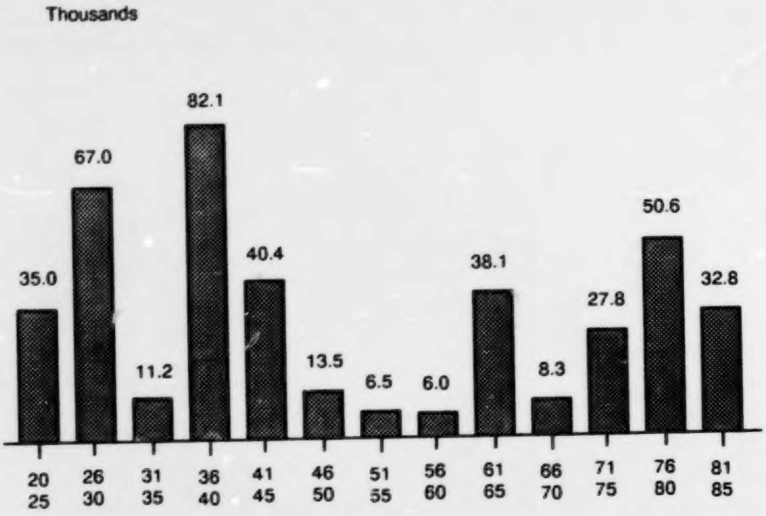


Figure 1.1.2. Cases of waterborne disease, 1920-1985.

Table 1.1.1. Illness and Deaths Associated with Waterborne Outbreaks, 1920-1985

Time Period	Cases per Outbreak	Deaths
1920-25	254	435
1926-30	572	234
1931-35	100	238
1936-40	504	82
1941-45	206	55
1946-50	115	6
1951-55	125	3
1956-60	101	7
1961-65	681	13
1966-70	110	7
1971-75	224	2
1976-80	251	1
1981-85	186	0

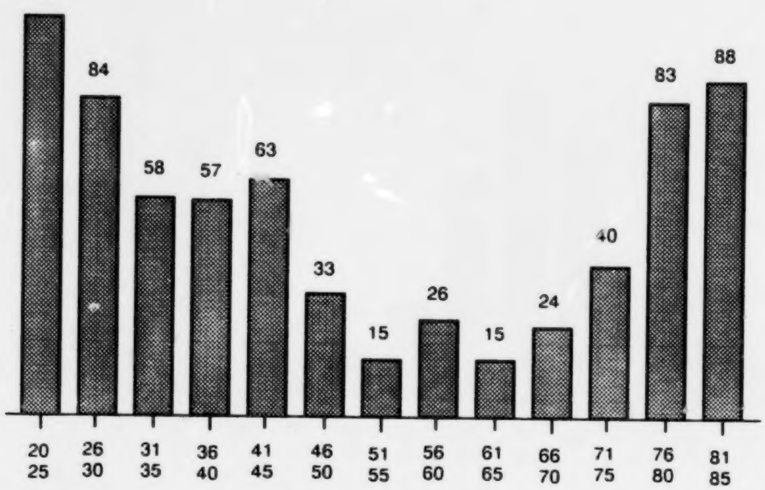


Figure 1.1.3. **Number of waterborne disease outbreaks in community water systems, 1920-1985.**

when the number of outbreaks reported was 27 and 18, respectively, compared with 48 reported in 1983. The decline in reported waterborne outbreaks was observed primarily in noncommunity systems. The number of outbreaks in noncommunity systems decreased from a total of 92 reported during 1976-1980 to 55 during 1981-1985, whereas the number of outbreaks reported in community systems was similar for each of these periods (Figures 1.1.3 and 1.1.4). Annually, the number of outbreaks reported in noncommunity systems decreased from 18 in 1981 to 8 in 1983, 5 in 1984, and 9 in 1985. In community systems, the number of reported outbreaks increased from 14 in 1981 to 30 in 1983, but then decreased to 12 in 1984 and 6 in 1985.

Outbreaks in community water systems, which number about 59,000 and serve about 180 million people, are probably the most likely to be reported. Because less active surveillance and reporting should continue to recognize and investigate potential outbreaks in at least the larger community water systems, perhaps the decrease in outbreaks observed in 1985 is a result of improvements in these water systems. Additional data for 1986-1988 are required before a trend can be established. Outbreaks in noncommunity systems, which number about 240,000 and serve about 20 million people, primarily transients, are the next most likely to be reported. However, an active surveillance program would be required to continue to detect these

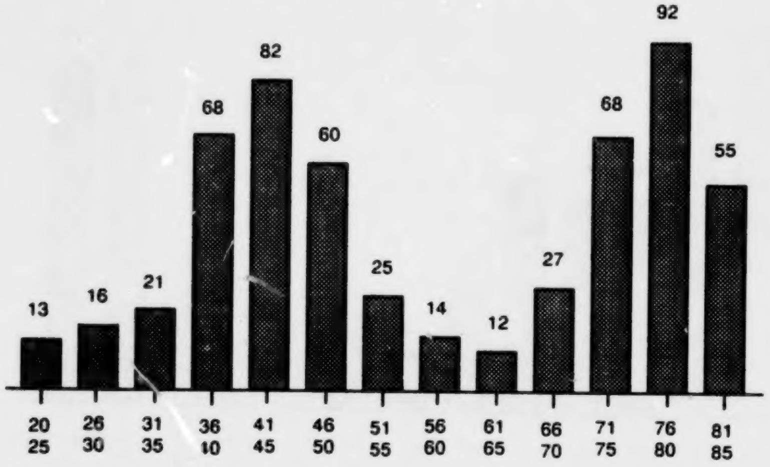


Figure 1.1.4. Number of waterborne disease outbreaks in noncommunity water systems, 1920-1985.

Table 1.1.2. Waterborne Outbreaks by Type of Water System, 1971-1985

Community	211	75,754
Noncommunity	216	33,958
Individual	59	719
Recreational	16	797
Totals	502	111,228

outbreaks, especially those involving travelers. Therefore, the drastic decline in outbreaks observed for noncommunity water systems since 1981 may be primarily the result of less active reporting and may not reflect an actual decrease in outbreak occurrence. Outbreaks in individual and recreational water systems are the least likely to be reported, and their recognition is, therefore, most susceptible to surveillance trends.

During the previous 15-year period, the number of reported outbreaks increased most dramatically during 1979-1983; it could also be argued that this large increase was primarily the result of improved surveillance and reporting, while the decrease in reported outbreaks observed since 1983 reflected a less active, more typical surveillance system. The numbers of reported outbreaks in 1984 and 1985 are comparable to the number of outbreaks reported each year during the period 1967-1975. An annual average of 22.5 outbreaks was reported during 1984-1985 compared with 21.3 during 1967-1975.

Many factors influence the degree to which outbreaks are recognized, investigated, and reported in any single year, including interest in the problem and the capabilities for recognition and investigation at the state and local level. For example, officials in Pennsylvania, Colorado, and Washington generally increased their waterborne disease surveillance activities during the 1970s; together these states reported 31 percent of all waterborne outbreaks during the period 1971-1980. During the previous 10-year period, only 8 percent of all waterborne outbreaks were reported by these states (15). Examination of the period 1981-1985 shows that these three states reported 44 percent of all waterborne outbreaks during 1981-1983 but only 20 percent of all waterborne outbreaks during 1984-1985. When three states report almost one third to one half of all waterborne outbreaks during a period of time, it is difficult not to attribute an increased number of outbreaks primarily to improved surveillance activities. It will be interesting to see if decreases continue to be observed in both reported outbreaks and the total proportion of outbreaks reported by these states.

A similar situation occurred during 1941-1950, when the large number of reported waterborne outbreaks was attributed primarily to an active surveillance program in a single state (15). New York reported 46 percent of all waterborne outbreaks during 1941-1950 compared with 18 percent during the previous 21-year period and 7 percent during the subsequent 30-year period. One cannot be certain whether the changes in the number of waterborne outbreaks reported in these states over various time periods reflect an actual decrease or increase in outbreaks or merely differences in surveillance and reporting activities. However, the magnitude of the changes observed in outbreaks reported by these few states suggests the importance of surveillance differences during the various time periods, and one should be cautious about ascribing increases or decreases in reported waterborne outbreaks to the deterioration or improvement of water systems.

While it is generally agreed that waterborne outbreak reporting is incomplete, it is difficult to estimate the number of outbreaks that may go undetected or unreported. One estimate, based on data collected from 1946 to 1970, suggested that about one half of the waterborne outbreaks in community water systems and about one third of those in noncommunity systems are detected, investigated, and reported (17). During a 3-year period of intensive waterborne disease surveillance in

Colorado from 1980 to 1983, 18 waterborne outbreaks were documented compared to 6 waterborne outbreaks during the previous 3-year period when the health department had a passive waterborne outbreak surveillance program (18,19). A study of the occurrence of foodborne outbreaks in Washington State indicated that prior to initiation of improved surveillance and investigation only 1 foodborne outbreak in 10 had been recognized and reported (20). Although this study considered only the reporting of foodborne outbreaks, the results may be applicable to waterborne outbreaks, especially those that occur in noncommunity water systems because of the similarities in methods of recognition and investigation of these kinds of outbreaks. This estimate could represent the maximum number of unreported waterborne outbreaks in small water systems.

In Colorado, increased disease surveillance and follow-up were found to be more effective in detecting waterborne outbreaks than increased surveillance and follow-up of water quality problems (18,19). Activities important for effective surveillance included educational outreach programs to local health agencies, physicians, and the public and the designation of one individual to whom all water-related complaints and health department inquiries were directed.

A waterborne outbreak (21) in a residential community of 6,500 persons in Florida in 1974 is a good example of how an active disease surveillance program can help detect outbreaks. Initially, only 10 cases of shigellosis were recognized by health authorities, but further investigation detected an additional 1,200 illnesses. If local health authorities had not been conducting shigellosis surveillance, the initial 10 cases might never have been recognized as an unusual occurrence, and an outbreak of waterborne disease as large as this might have gone undetected.

Causes of Outbreaks

The use of contaminated, untreated, or inadequately disinfected ground water was responsible for 49 percent of the waterborne outbreaks (Figure 1.1.5) and 47 percent of the cases of illness (Figure 1.1.6) reported during 1971-85. The use of contaminated, untreated, or inadequately treated surface water was responsible for 24 percent of the waterborne outbreaks and 32 percent of the cases. The remaining outbreaks were caused by contamination of the distribution system and miscellaneous deficiencies, which include use of water not intended for consumption, contaminated ice, and ingestion of contaminated water while swimming. Also included under miscellaneous deficiencies were 15 outbreaks where data were insufficient to determine the source of contamination.

When waterborne outbreaks caused by *Giardia* are analyzed separately, it becomes clear that water supply deficiencies responsible for giardiasis are different from the deficiencies responsible for other

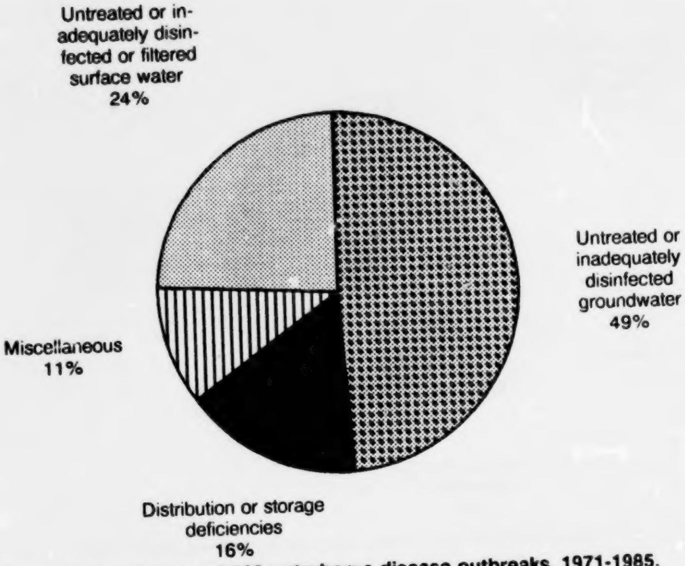


Figure 1.1.5. Causes of 502 waterborne disease outbreaks, 1971-1985.

diseases (Figure 1.1.7). Over 70 percent of the waterborne outbreaks of giardiasis during 1971-1985 were attributed to no or inadequate treatment of surface water; 20 percent of the giardiasis outbreaks were attributed to use of contaminated, untreated ground water, inadequate or interrupted disinfection of ground water, cross-connections, and other distribution system deficiencies. For all other waterborne diseases reported during 1971-1985, only 14 percent of the outbreaks were attributed to no or inadequate treatment of surface water; the majority (74 percent) were attributed to contaminated, untreated, and inadequately disinfected ground water, cross-connections, and other distribution deficiencies.

Causes of Outbreaks in Ground-Water Systems

Contaminated ground water has consistently been responsible for more waterborne outbreaks than contaminated surface water. In each time period since 1930, between 42 and 56 percent of all outbreaks were caused by use of contaminated ground water; 14 to 27 percent of all outbreaks were caused by contaminated surface water.

During 1971-1985, the use of untreated or inadequately treated ground water resulted in 243 outbreaks and 52,091 cases of illness. A

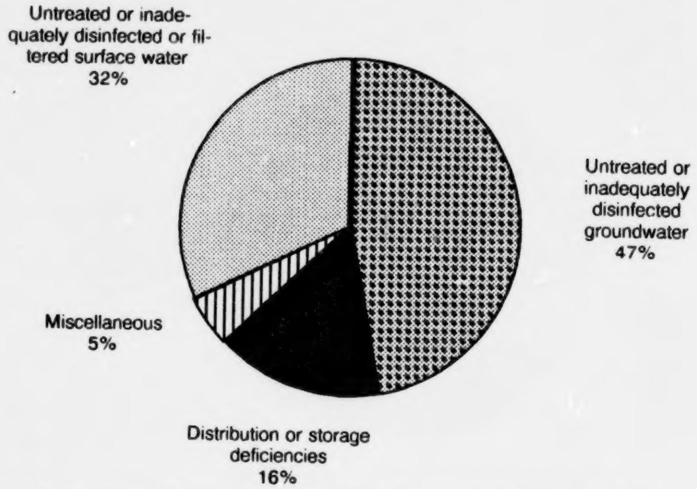


Figure 1.1.6. Causes of 111,228 waterborne disease outbreaks, 1971-1985.

seasonal distribution of outbreaks in these systems is apparent; 71 percent occurred during May through September.

As a cause of outbreaks, use of contaminated, untreated ground water has decreased in importance while inadequate or interrupted disinfection of ground water has increased (Figure 1.1.8). For example, use of contaminated, untreated ground water was responsible for 53 percent of all waterborne outbreaks during 1931-1940 but only 29 percent during 1981-1985. Inadequate or interrupted disinfection caused 21 percent of all outbreaks during 1971-1980 compared with 2 percent during 1920-1930. The increased use of disinfection, especially in situations where little or no effort has been made to reduce groundwater contamination, and the lack of attention to maintaining adequate, continuous disinfection have likely been responsible for the increased outbreaks in disinfected ground-water systems. Most of the outbreaks in disinfected ground waters were the result of improper chlorination; however, several outbreaks during 1971-1985 resulted from inadequate or interrupted iodine disinfection in noncommunity systems.

Some 25 percent of the outbreaks in untreated ground-water systems during 1971-1985 were caused by the overflow or seepage of sewage into wells and springs; 13 percent were caused by surface runoff or flooding from contaminated streams; 8 percent were caused

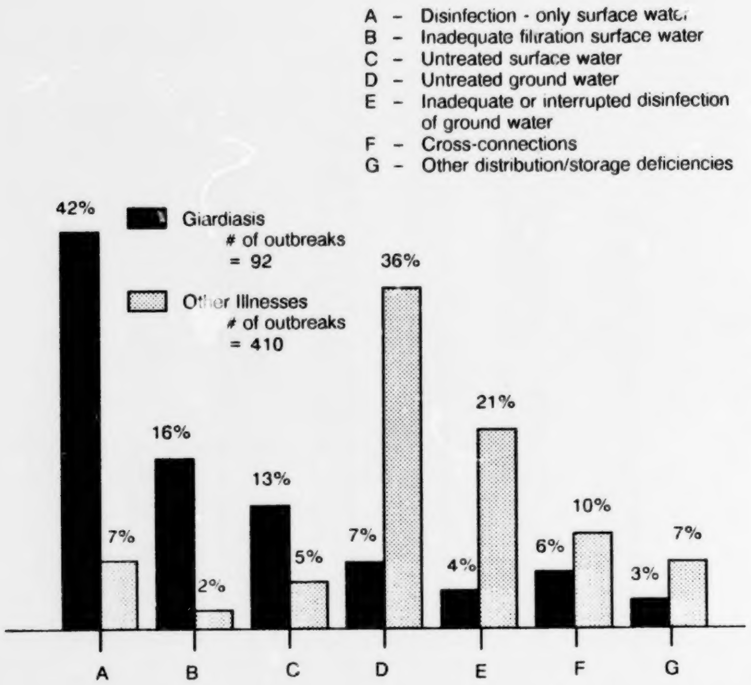


Figure 1.1.7. **Major water supply deficiencies responsible for waterborne disease outbreaks, 1971-1985.**

by chemical contamination; and 5 percent were caused by contamination through limestone or fissured rock. Improper system construction was identified in 3 percent of the outbreaks, and there were insufficient data to classify the remaining outbreaks in untreated ground-water systems. Contaminants detected in ground waters in cases of chemical poisoning were nitrate, benzene, phenol, selenium, trichloroethylene, polychlorinated biphenyls, oil, and gasoline.

Causes of Outbreaks in Surface Water Systems

One hundred twenty-three waterborne outbreaks during 1971-1985 were attributed to the use of contaminated, untreated, or inadequately treated surface water. A seasonal distribution of outbreaks in these systems is also apparent; 60 percent occurred

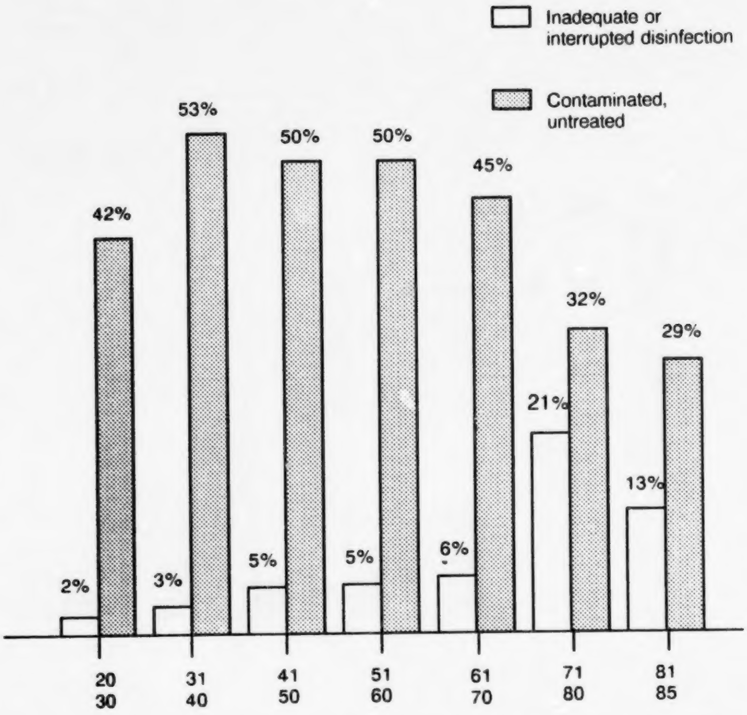


Figure 1.1.8. **Waterborne outbreaks in untreated and disinfected only groundwater systems (wells and springs), 1920-1985.**

during May through September. The numbers of outbreaks occurring in untreated, disinfected only, and filtered surface water systems were also examined separately to determine the relative importance of each of these deficiencies over the years. The increased use of disinfection and filtration for surface waters since 1920 is the likely explanation for the decreased importance of contaminated, untreated surface water in the transmission of waterborne disease (Figure 1.1.9). For example, 19 percent of waterborne outbreaks during 1920-1930 were attributed to untreated surface water compared with 3 percent during 1981-1985.

Until 1971, more outbreaks were attributed to the use of contaminated, untreated surface water than to deficiencies in the treatment of surface water. Now, outbreaks in surface water systems occur primarily because of inadequate or interrupted disinfection, especially in systems which provide disinfection as the only treatment (Figure 1.1.9). Thirteen percent and 14 percent of waterborne

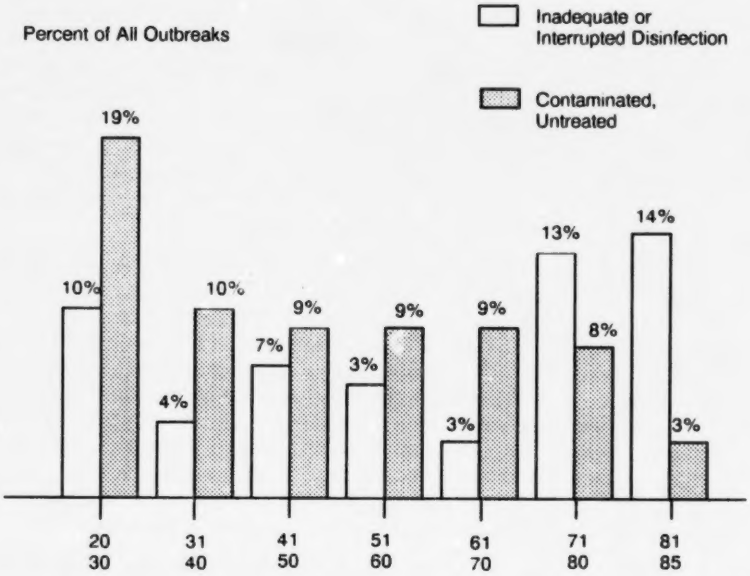


Figure 1.1.9. **Waterborne outbreaks in untreated and disinfected only surface water systems, 1920-1985.**

outbreaks during 1971-1980 and 1981-1985, respectively, were attributed to inadequate or interrupted disinfection of disinfected only surface water; previously only 3 to 7 percent of waterborne outbreaks had been attributed to this deficiency since 1931. In recent years, an increased number of waterborne outbreaks of giardiasis has occurred in unfiltered surface water sources in which disinfection was inadequate or interrupted. Disinfection with chlorine can inactivate pathogens including *Giardia* cysts only if several conditions are maintained:

- The water consistently has low concentrations of substances which can cause turbidity, create a chlorine demand, or interfere with the disinfection process.
- A sufficient concentration of disinfectant and contact time, as determined from laboratory or field studies, are provided according to various operating parameters, especially water temperature and pH.
- The chlorine residual is not depleted for any reason.

- Heavy or overwhelming contamination does not occur either briefly or intermittently.

In the giardiasis outbreaks reported in unfiltered systems having inadequate disinfection, one or more of these conditions were not met. In most outbreaks, the concentration of chlorine and/or contact time provided was insufficient; in all outbreaks disinfection was ineffective for the inactivation of pathogens in the raw water. In several outbreaks, it was noted that during the period of suspected contamination, chlorine residual levels were reduced to nil. In outbreaks where disinfection was interrupted, chlorine equipment failed or the chlorine supply was exhausted.

In filtered surface water systems, the number of outbreaks attributed to inadequate or interrupted disinfection has remained relatively stable over the years, causing 1 to 2 percent of waterborne outbreaks. During 1981-85, a dramatic increase in outbreaks was noted for filtered surface water supplies; 8 percent of all waterborne outbreaks during that period were attributed to inadequate control of filtration or pretreatment. This increased number of outbreaks in filtered water systems serves as a reminder that treatment not only must be appropriately designed to deal with varying source water quality but also must be properly operated and maintained to provide continuous and effective removal and inactivation of pathogens.

Data have recently been made available on the number of communities and populations served by filtered and unfiltered surface water sources (16). These data were combined with the data on waterborne outbreaks to obtain outbreak and disease rates for various water sources and treatment. Comparison of these rates can provide an indication of the susceptibility of water systems. Outbreak and disease rates were calculated using the waterborne outbreaks reported during 1971-1985 in community water systems with surface water sources (Figures 1.1.10 and 1.1.11). Outbreaks caused by contamination of the distribution system and miscellaneous deficiencies in surface water systems were excluded, since their occurrence is unrelated to source water contamination and treatment. The reported chemical poisonings caused by deficiencies in chemical feed were also excluded. Because the reporting of waterborne outbreaks is voluntary, the rates should be used primarily for comparison purposes. For identifying water systems with the greatest potential for failure, the outbreak rate (Figure 1.1.10) is less variable and represents a more stable rate for comparison purposes. However, the disease rate (Figure 1.1.11) may have more public health significance because the prevention of large outbreaks can result in a dramatically lower disease rate.

Approximately 4,611 community water systems serve filtered and disinfected surface water to a population of some 133,560,000. These systems experienced 23 outbreaks and 9,450 cases of illness for a rate

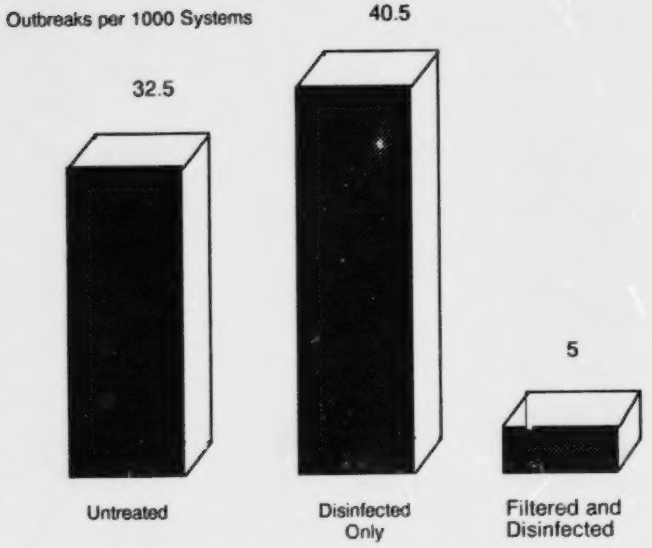


Figure 1.1.10. Waterborne outbreak rates attributed to source contamination and treatment inadequacies in community systems using surface water sources, 1920-1985.

of 5.0 waterborne outbreaks per 1,000 facilities served during 1971-1985 and 4.7 cases of waterborne illness per million person-years. Community water systems that provide disinfection as the only treatment for surface water experienced an outbreak rate of 40.5/1,000 facilities (8 times the rate for filtered surface water) and a disease rate of 66.3/million person-years (14 times the rate for filtered surface water). The rates for community water systems using untreated surface water are also much higher than the rates for communities with filtered surface water; however, these rates are based on only five outbreaks and 612 cases of illness in 154 water systems serving 110,000 people.

Etiologic Agents

An etiologic agent was determined in only 50 percent of all waterborne outbreaks during 1971-85; the remaining outbreaks were categorized as acute gastrointestinal illness of unidentified etiology (Figure 1.1.12). In ground-water systems, an agent was identified in only 38 percent of the outbreaks; in surface water systems, an agent was identified in 62 percent of the outbreaks. In many of the outbreaks of undetermined etiology, the search for an etiologic agent included

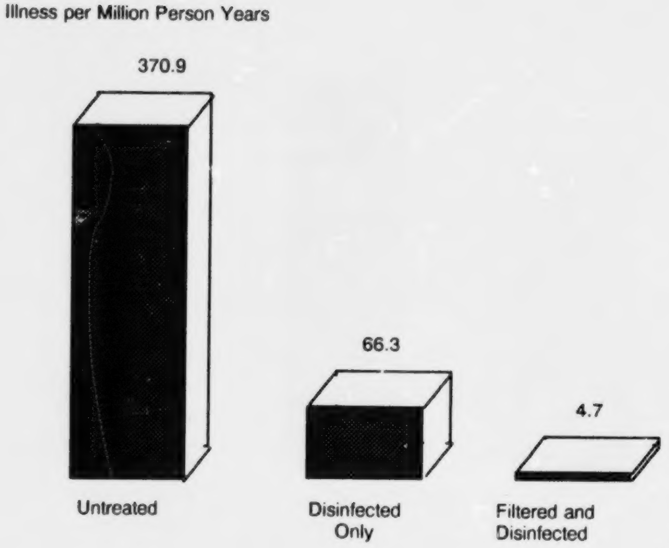


Figure 1.1.11. Waterborne disease rates attributed to source contamination and treatment inadequacies in community systems using surface water sources, 1971-1985.

only stool cultures for *Salmonella* or *Shigella*; in others, the investigation and collection of clinical specimens were delayed, and the etiologic agent could not be identified because samples were not collected in a timely manner. It is suspected that the gastroenteritis outbreaks represent a combination of viral, bacterial, and parasitic etiologies.

In recent years waterborne viral gastroenteritis caused by Norwalk agent or rotavirus, bacterial gastroenteritis caused by *Campylobacter* and *Yersinia*, giardiasis, and cryptosporidiosis have been reported in both surface and ground-water systems. During a waterborne outbreak, each of these agents should be considered, depending upon the symptomatology, and appropriate clinical testing should be performed to identify the etiologic agent. Only by identifying the etiologic agents of waterborne outbreaks can researchers ascertain whether current surveillance activities, regulations, and water treatment techniques are adequate to prevent the waterborne transmission of specific infectious diseases.

Although each of these etiologic agents could be transmitted through either contaminated ground-water or surface water systems, *Giardia lamblia* was found to be the most commonly identified

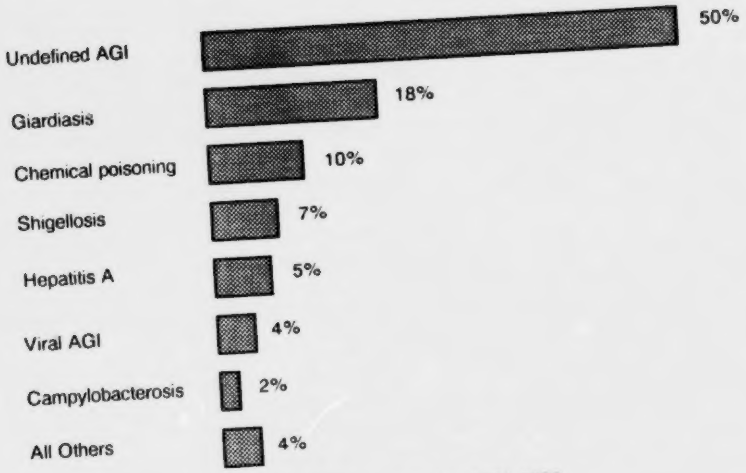


Figure 1.1.12. Etiology of waterborne outbreaks, 1971-1985.

pathogen responsible for waterborne outbreaks in surface water systems. Shigella and hepatitis A virus were the two most commonly identified pathogens in ground-water outbreaks. Almost all of the outbreaks of hepatitis in ground-water systems were caused by sewage contamination of untreated wells and springs, while shigellosis outbreaks in ground-water systems were caused both by sewage contamination of untreated water sources and interrupted or inadequate disinfection of contaminated wells and springs. Outbreaks of chemical poisoning occurred when ground-water sources were contaminated and through cross connections and inadequate control of chemical feed in surface water systems. Of the 50 reported outbreaks of chemical poisonings, 13 resulted from contamination of wells and springs; 12 from cross connections; 12 from corrosion or other distribution deficiencies; and 8 from deficiencies in the feeding of chemicals during treatment.

Giardiasis was the disease most often transmitted because of ineffective filtration or pretreatment of surface water and inadequate disinfection of surface water when disinfection was the only treatment. These two deficiencies were responsible for 56 percent of all cases of illness reported in surface water systems. While a significant number of giardiasis outbreaks occurred in untreated surface water systems, the systems affected were small and only 322 cases of giardiasis were reported. Although contamination of source waters by human sewage has caused waterborne outbreaks of giardiasis, numerous wild and domestic animals, especially beavers, have been implicated as primary or intermediary sources of infection and also important in waterborne transmission (22,23). Because animals cannot be completely excluded from the watershed, all surface water supplies are at risk of contamination with *Giardia*. Restricting discharges of human sewage on the watershed may be insufficient protection for surface water systems that only disinfect, and additional treatment barriers may be needed to prevent the waterborne transmission of giardiasis. Outbreak rates have shown the importance of surface water filtration, but greater emphasis is also needed on the design, installation, operation, and maintenance of filtration facilities. Giardiasis outbreaks have also occurred in ground-water systems where human sewage or contaminated surface water entered improperly constructed or located wells. Six giardiasis outbreaks were reported in untreated ground-water systems and four were reported in disinfected ground-water systems.

Only one outbreak of cryptosporidiosis was reported during 1971-1985. This outbreak occurred in a ground-water system, but *Cryptosporidium* could be an important contaminant of surface water systems as well. The occurrence of the protozoan is widespread, and animals, as well as humans, are involved in the waterborne transmission of this organism (24,25). Little is known about the inactivation of *Cryptosporidium* oocysts by chemical disinfectants, but the available data suggest the organism may be more resistant to chlorination than Giardia (26). Thus, it may be necessary to remove the oocysts from water by filtration rather than depend upon inactivation by disinfection.

A second large waterborne outbreak of several thousand cases of cryptosporidiosis occurred in a filtered surface water system in Carrollton, Georgia, during January and February 1987. Water treatment in this system included coagulant feed, mechanical rapid mix, flocculation, sedimentation, and filtration through anthracite sand filters (2 gallons per minute per square foot) (27). Turbidity was not routinely monitored at each filter effluent, but was measured during the outbreak investigation. Analysis of the filtered water turbidity of each filter's effluent suggested that the practice of stopping and later restarting the flow of water through some filters without backwashing could result in passage of alum floc and contaminants through the filters into the finished water. Earlier EPA research indicated that turbidity breakthrough, or passage of floc through the filter, could be accompanied by contaminants such as *Giardia* cysts. *Cryptosporidium* may have entered the system because filters were not backwashed prior to placing them in service.

Summary

Waterborne outbreaks continue to occur, and the largest number in any 15-year period since 1920 was reported during 1971-1985. However, the reporting of waterborne outbreaks has declined since

1980. Since outbreaks now occur primarily in small community and noncommunity water systems, the incidence of waterborne disease has declined since 1920. Almost three fourths of the outbreaks in the past 15 years were caused by contaminated, untreated, or inadequately treated ground water and surface water.

EPA regulations were found insufficient to prevent the waterborne transmission of infectious disease, as waterborne outbreaks have occurred in systems which had not exceeded current EPA regulations for coliforms and turbidity. In addition to the protection of source water quality, adequate, continuous disinfection is a necessary final barrier to prevent the transmission of waterborne disease. Disinfection has been inadequate as the only treatment for surface water. In all but exceptional situations, effective filtration must also be provided (including any required pretreatment) to ensure the removal and inactivation of waterborne pathogens, especially *Giardia* and *Cryptosporidium*. Outbreaks have occurred in water systems that both filter and disinfect, when facilities have been improperly designed or have been operated in a poor or simply casual manner, thus emphasizing the need to maintain effective and continuous water treatment. To reduce the occurrence of waterborne outbreaks, EPA has promulgated regulations for filtration of surface water supplies and disinfection of all water sources, and revised regulations for coliforms (28,29). More emphasis should also be placed on the design and operation of filtration facilities, if they are to be an effective barrier against the transmission of waterborne disease.

While the protection of raw water quality continues to be important, it can only be considered one barrier to the transmission of waterborne disease, especially with increasing evidence of the importance of domestic and wild animals in waterborne disease transmission. The increasing potential for contamination of surface water sources warrants the maintenance of additional barriers, including filtration and disinfection, to ensure adequate margins of safety. The multiple barrier concept has evolved from water treatment experiences, and, if properly applied to surface water systems, can significantly reduce the risk of waterborne transmission of infectious disease.

Disinfection may be the final barrier against transmission of pathogens, but it must not be the only one. Properly designed and operated filtration plants can make disinfection more effective by removing turbidity and some substances that exert chlorine demand and reducing microbiological contamination (30-32). If disinfection is used as one part of the multiple barrier concept and not relied upon so heavily, lower concentrations of chlorine can be used for inactivation of pathogens in filtered water, thereby lowering concentrations of chlorinated by-products.

References

1. Foodborne Outbreaks Annual Summary 1972, Centers for Disease Control, Department of Health, Education and Welfare, Publication No. (CDC)74-8185, 1973.
2. Foodborne and Waterborne Disease Outbreaks Annual Summary 1973, Centers for Disease Control, Department of Health, Education and Welfare, Publication No. (CDC)75-8185, 1974.
3. Foodborne and Waterborne Disease Outbreaks Annual Summary 1974, Centers for Disease Control, Department of Health, Education and Welfare, Publication No. (CDC)76-8185, 1976.
4. Foodborne and Waterborne Disease Outbreaks Annual Summary 1975, Centers for Disease Control, Department of Health, Education and Welfare, Publication No. (CDC)76-8185, 1976.
5. Foodborne and Waterborne Disease Outbreaks Annual Summary 1976, Centers for Disease Control, Department of Health, Education and Welfare, Publication No. (CDC)78-8185, 1977.
6. Foodborne and Waterborne Disease Surveillance Annual Summary 1977, Centers for Disease Control, Department of Health, Education and Welfare, U.S. Government Printing Office 640-010-3610, 1979.
7. Water-Related Disease Outbreaks Surveillance Annual Summary 1978, Centers for Disease Control, Health and Human Services Publication No. (CDC)80-8385, 1980.
8. Water-Related Disease Outbreaks Surveillance Annual Summary 1979, Centers for Disease Control, Health and Human Services Publication No. (CDC)81-8385, 1981.
9. Water-Related Disease Outbreaks Surveillance Annual Summary 1980, Centers for Disease Control, Health and Human Services Publication No. (CDC)82-8385, 1982.
10. Water-Related Disease Outbreaks Surveillance Annual Summary 1981, Centers for Disease Control, Health and Human Services Publication No. (CDC)82-8385, 1982.
11. Water-Related Disease Outbreaks Surveillance Annual Summary 1982, Centers for Disease Control, Health and Human Services Publication No. (CDC)83-8385, 1983.
12. Water-Related Disease Outbreaks Surveillance Annual Summary 1983, Centers for Disease Control, Health and Human Services Publication No. (CDC)84-8385, 1984.
13. Water-Related Disease Outbreaks Surveillance Annual Summary 1984, Centers for Disease Control, Health and Human Services Publication No. (CDC)99-2510, 1985.

14. CDC Surveillance Summaries, Morbidity and Mortality Weekly Report, Health and Human Services Publication No. (CDC)88-8017, 1988.

15. Craun, G.F. Statistics of waterborne outbreaks in the U.S. (1920-80) *In:* G.F. Craun (ed.), Waterborne Diseases in the United States, CRC Press, Inc., Boca Raton, FL, 1986, p. 73.

16. Craun, G.F. Surface water supplies and health. *J. AWWA.* 80:40, 1988.

17. Craun, G.F. and McCabe, L.F. Review of the causes of waterborne disease outbreaks. *J. AWWA.* 65:74, 1973.

18. Hopkins, R.S., Shillam, P., Gaspard, B., Eisnach, L., and Karlin, R.J. Waterborne disease in Colorado: three years' surveillance and 18 outbreaks. *Am. J. Public Health.* 75:254, 1985.

19. Harter, L., Frost, F., Vogt, R., Lettle, A.A., Hopkins, R., Gaspard, B., and Lippy, E.C. A three-state study of waterborne disease surveillance techniques. *Am. J. Public Health.* 75:1327, 1985.

20. Barker, W.H., Jr. Foodborne disease surveillance-Washington state, *Am. J. Public Health.* 64:26, 1974.

21. Weisman, J.B., Craun, G.F., Lawrence, D.N., Pollard, R.A., Saslaw, M.S., and Gangarosa, E.J. An epidemic of gastroenteritis traced to a contaminated public water supply. *Am. J. Epidemiol.* 103:391, 1976.

22. Craun, G.F. Waterborne outbreaks of giardiasis. *In:* S. L. Erlandsen and E. A. Meyer (ed.), Giardia and Giardiasis. Plenum Press, New York, 1984, p. 243.

23. Craun, G.F. and Jakubowski, W. Status of waterborne giardiasis outbreaks and monitoring methods. *In*: C.L. Tate, Jr. (ed.), Proceeding International Symposium on Water Related Health Issues. Am. Water Resources Assoc., Bethseda, MD, p. 167.

24. Navin, T.R. and Juranek, D.D. Cryptosporidiosis: clinical, epidemiologic, and parasitologic review. *Reviews Infect. Diseases.* 6:313, 1984.

25. Tzipori, S. Cryptosporidiosis in animals and humans. *Microbiol. Reviews.* 47:84, 1983.

26. Hoff, J.C., Inactivation of waterborne protozoan cysts. American Society for Microbiologists Conference. Atlanta, GA, March 3, 1987.

27. Hayes, E.B. *et al.* Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. *N. Engl. J. Med.* 320:1372, 1989.

28. Drinking water: National primary drinking water regulations; filtration, disinfection; turbidity, *Giardia lamblia*, viruses, *Legionella*, and heterotrophic bacteria. Proposed rule. 40 CFR parts 141 and 142, *Fed. Reg.* 52(212):42718, 1987.

29. National primary drinking water regulations: total coliforms. Proposed Rule. 40 CFR parts 141 and 142. *Fed. Reg.* 52(212):42224, 1987.

30. Logsdon, G.S. and Hoff, J.C. Barriers to the transmission of waterborne disease. In: G.F. Craun (ed.), Waterborne Diseases in the United States. CRC Press, Inc., Boca Raton, FL, 1986, p. 255.

31. Logsdon, G.S., Thurman, V.C., Frindt, E.S., and Stoecker, J.G. Evaluating sedimentation and various filter media for removal of Giardia cysts. *J. AWWA.* 77:61, 1985.

32. Logsdon, G.S. and Rice, E.W. Evaluation of sedimentation and filtration for microorganism removal. *In:* Proceedings Annual Conference, American Water Works Association, Denver, CO, June 23-27, 1985, p. 1177.

The Safe Drinking Water Act and the Regulation of Microorganisms in Drinking Water

by: Paul S. Berger and Stig Regli
Office of Drinking Water
U.S. Environmental Protection Agency
Washington, DC 20460
(202) 382-3039
(202) 382-7379

Requirements of the Safe Drinking Water Act

The Safe Drinking Water Act (SDWA) requires the U.S. Environmental Protection Agency (EPA) to publish National Primary Drinking Water Regulations (NPDWRs) which set either a maximum contaminant level (MCL) or a water treatment technique requirement for contaminants that may have an adverse health effect. The SDWA also requires EPA to specify monitoring and reporting requirements for each regulated contaminant.

The SDWA requires EPA to set both a maximum contaminant level goal (MCLG) and a NPDWR for each contaminant. The MCLG is a nonenforceable health goal that is set at a level at which no known or anticipated adverse health effects occur and which allows an adequate margin of safety. In contrast, the MCL, if set, is an enforceable standard which is set as close to the MCLG as is feasible, taking cost and other factors into account.

According to the SDWA, EPA is to set an MCL for a contaminant if it is economically and technologically feasible to ascertain the level of the contaminant. If not, EPA is to set a water treatment requirement. Some of the factors EPA considers in making this judgment include method reliability, laboratory experience with available methods, method detection limits, the ability to relate the measurement to the determination of health risk significance, and cost of analysis.

EPA currently regulates two indicators of microbiological drinking water quality: total coliforms and turbidity. The 1986 Amendments to the SDWA require EPA to regulate *Giardia lamblia*, viruses, *Legionella*, and heterotrophic bacteria, as well as total coliforms and turbidity. They also require EPA to publish regulations specifying criteria under which filtration is required as a treatment technique for public water systems supplied by surface water sources. In addition, the 1986 amendments require the Agency to publish regulations requiring disinfection as a treatment technique for all public water systems (except those which meet certain criteria allowing them to avoid this requirement).

EPA will comply with the above requirements of the 1986 amendments by publishing three rulemaking packages: the revised total coliform rule, the surface water treatment requirements (SWTR), and the ground-water disinfection rule. As of the date of publication of these proceedings, EPA has published the revised total coliform rule and the SWTR. The Agency believes that, collectively, the requirements in these three packages will minimize occurrence of waterborne disease in the United States.

Surface Water Treatment Requirements (SWTR)
(54 FR 27486-27541, June 29, 1989)

Under the SWTR, all public water systems using surface water or ground water under the direct influence of surface water must provide disinfection. Systems must also filter the water unless they meet specific conditions relating to source water quality. The SWTR will also regulate *Giardia lamblia*, turbidity, viruses, heterotrophic bacteria, and *Legionella* in surface waters.

The SWTR will regulate *Giardia lamblia*, turbidity, viruses, heterotrophic bacteria, and *Legionella* by establishing treatment technique requirements rather than maximum contaminant levels. Under the rule, all systems using surface water must achieve at least 99.9 percent removal/inactivation of *Giardia lamblia* cysts and 99.99 percent removal/inactivation of viruses.

Criteria Systems Must Meet to Avoid Filtration

To avoid filtration, systems must meet the following criteria:

- **Measure turbidity levels every 4 hours.** The turbidity level must not exceed 5 NTU.
- **Test the source water for either total coliforms or fecal coliforms at least once per week to as much as to 5 per week (depending on population served).** Also, during days that the turbidity exceeds 1 NTU in the raw water, at least one total or fecal coliform measurement must be made. Total coliforms must not exceed 100/100 mL or fecal coliforms must not exceed 20/100 mL in more than 10 percent of the measurements for the previous 6 months, calculated each month.
- **Maintain a disinfectant residual concentration of at least 0.2 mg/L in water entering the distribution system, as demonstrated by continuous monitoring.** Systems serving 3,300 persons or less can take one to four grab samples (depending on population served) instead of continuous

monitoring. Provide disinfection to ensure that the 99.9 percent and 99.99 percent removal/inactivation of *Giardia lamblia* and viruses, respectively, are being achieved as demonstrated by meeting C·T values in the rule (C·T is the product of residual concentration in mg/L and disinfectant contact time in minutes, measured at peak hourly flow).

- **Maintain a detectable disinfectant residual in the distribution system.** Disinfectant residuals cannot be undetectable in more than 5 percent of the samples each month, for 2 consecutive months. Samples must be taken at the same frequency as total coliform samples. A system may measure heterotrophic bacteria (HPC) in lieu of disinfectant residual. If the HPC is less than 500 colonies/mL, the site is considered to be equivalent to having a detectable disinfectant residual.
- **Maintain a watershed control program that will minimize the potential for contamination by human enteric viruses and *Giardia lamblia* cysts.**
- **Have had no waterborne disease outbreaks in its present configuration.** If an outbreak occurs, system must either filter or correct the deficiency to the state's satisfaction to prevent another such outbreak.
- **Be in compliance with the MCL for total coliforms during at least 11 of 12 consecutive months**, unless the state determines the violations are not due to a treatment deficiency.
- **Be in compliance with the MCL for total trihalomethanes.** Currently, this requirement only pertains to systems serving greater than 10,000 people. When the regulations for disinfection by-products are promulgated, EPA will require compliance for all system sizes as a condition for avoiding filtration.

Criteria Filtered Systems Must Meet

Filtered systems must measure turbidity levels every 4 hours or by continuous monitoring. The state may reduce the number of grab samples to once per day for some systems. For systems using conventional filtration or direct filtration, the turbidity levels in filtered water must never exceed 5 NTU and not exceed 0.5 NTU in more than 5 percent of the samples collected each month. (The state may allow a level up to but less than 1 NTU under certain conditions.) For systems using slow sand filtration or diatomaceous earth, the turbidity level in the filtered water must always be less than 5 NTU and not exceed 1 NTU in more than 5 percent of the samples collected each month. (The state may allow a system using slow sand filtration to exceed 1 NTU under certain conditions.)

A filtered system must also monitor disinfectant residuals at the point of entry to the distribution system at the same frequency and locations as an unfiltered surface water system. Filtration and disinfection combined must achieve a 99.9 percent and 99.99 percent removal/inactivation of *Giardia lamblia* and viruses, respectively. The state defines the level of disinfection required.

Total Coliform Rule Requirements
(54 FR 27544-27568, June 29, 1989)

Maximum Contaminant Level (MCL)

The MCL is based on the percentage of samples collected during a month which contains any total coliforms, not on the density of total coliforms in a sample. For systems analyzing at least 40 samples/month, no more than 5.0 percent of the monthly samples may be total coliform-positive. For systems analyzing fewer than 40 samples/month, no more than one sample/month may be total coliform-positive.

Routine Monitoring

Each public water system must sample according to a written sample siting plan. The state must establish a process that ensures the adequacy of the sample siting plan for each system.

The monitoring frequency for community water systems is based upon the population served, as is the case under the current total coliform rule, but with only 34 rather than 84 population categories. For noncommunity water systems the monitoring frequency is as follows:

- A system using surface water, or ground water under the direct influence of surface water, must monitor at the same frequency as a like-sized community water system.
- A system serving more than 1,000 persons during any month must monitor at the same frequency as a like-sized community water system. The state may reduce the monitoring frequency for any month the system serves 1,000 persons or fewer.
- No system may monitor less than annually after June 1994.

Monitoring After a Total Coliform-Positive Sample

If a system has a total coliform-positive sample, it must collect a set of repeat samples within 24 hours after being notified. Systems collecting one routine sample/month or fewer must collect at least four repeat samples; all other systems must collect at least three repeat samples. Repeat samples must be taken 1) at the same tap as the original total coliform-positive sample, 2) at a tap within five service connections upstream, and 3) at a tap within five service connections downstream.

If any repeat sample is total coliform-positive, the system must collect another set of repeat samples, as before, unless the MCL has been violated and the system has notified the state.

If a system collects fewer than five routine samples/month, it must not only take a set of repeat samples within 24 hours, but also at least five routine samples the next month it provides water to the public. The state may waive the requirement for a system to collect five routine samples the next month under certain circumstances.

All total coliform samples - routine and repeat - count toward compliance, except those that are invalidated by the state (or, for total coliform- negative samples, by the laboratory).

If a routine or repeat sample is total coliform-positive, the system must analyze that total coliform-positive culture to determine if fecal coliforms or *Escherichia coli* are present. If present, the system must notify the state by the end of the next business day. If any repeat sample is fecal coliform- or *E. coli*-positive, or if a fecal coliform- or *E. coli*-positive routine sample is followed by a total coliform-positive repeat sample, the system is in violation of the MCL for total coliforms. When the MCL is violated under these circumstances, the system must notify the public via the electronic media.

Invalidation of Samples

A state may only invalidate a total coliform-positive sample under one of the following circumstances:

- The laboratory acknowledges it performed the analysis improperly.
- The system determines that the contamination is a nondistribution plumbing problem on the basis that any repeat sample taken at the same tap as the original total coliform-positive sample is also total coliform-positive, and all other repeat samples taken at nearby locations are total coliform-negative.
- The state has substantial grounds to believe that a coliform-positive result is due to some circumstance or condition not related to the quality of drinking water in the distribution system. In this case, the state must explain this judgment in writing, the supervisor of the state official who draws this conclusion must sign the document, and the document must be made available to EPA and the public. The written documentation must state the specific cause of the total

coliform-positive sample, and what action the system has taken, or will take, to correct this problem.

Laboratories must invalidate a total coliform-negative sample if there is evidence that high levels of heterotrophic bacteria interfered with the total coliform analysis.

Sanitary Surveys

All systems that collect fewer than five samples/month must conduct a periodic sanitary survey. The responsibility for conducting the sanitary survey is with the system, not the state.

A community water system must have a sanitary survey by June 29, 1994, and every 5 years thereafter. A noncommunity water system must have a sanitary survey by June 29, 1999, and every 5 years thereafter or, for systems that use protected and disinfected ground water, every 10 years thereafter.

Analytical Methodology

In conducting coliform monitoring, laboratories may use one or more of the following analytical methods: 10-tube multiple tube fermentation technique, membrane filter technique, presence-absence (P-A) coliform test, and minimal medium ONPG-MUG test. In addition, a 100-mL sample volume must be used in analyzing for total coliforms, regardless of the analytical method used.

II. Disease Surveillance

Waterborne Disease Outbreaks Surveillance: Federal Requirements and Responsibilities

by: Robert V. Tauxe, M.D., M.P.H.
Enteric Diseases Branch
Division of Bacterial Diseases
Center for Infectious Diseases
Centers for Disease Control
Atlanta, Georgia 30333
(404) 639-2888

Introduction

The current collaborative surveillance for waterborne disease outbreaks began at the federal level in 1971. Since then the U.S. Environmental Protection Agency (EPA) and Centers for Disease Control (CDC) have collected reports of waterborne disease outbreaks and have summarized them annually. The purposes of national surveillance of waterborne disease outbreaks have been 1) to determine general trends in the frequency of waterborne disease outbreaks, 2) to characterize the epidemiologic patterns of waterborne diseases, 3) to disseminate information on waterborne diseases, and 4) to provide data for evaluating disease control efforts.

For purposes of surveillance, a waterborne disease outbreak is defined as an acute illness affecting two or more persons with similar symptoms that is epidemiologically associated with ingestion of water or some other exposure to water intended for drinking. Single well-documented cases related to toxic exposures such as methemoglobinemia are also included. Reporting for all federal disease surveillance systems occurs as the result of a voluntary effort supported by the Council of State and Territorial Epidemiologists. Reporting of specific diseases is a result of legislation in each state concerning notifiable diseases; reporting of all diseases to the federal government is voluntary. In addition to outbreaks associated with water intended for drinking, CDC has also collected information on illness related to recreational water uses, and included these in annual summaries. The annual summaries have also included outbreaks of gastroenteritis on cruise ships, whether or not they were shown to be associated with water exposure. Outbreaks on cruise ships and outbreaks associated with ingestion of water while swimming have been excluded, however, from the statistics reported here.

Recent Trends

Between 1971 and 1985, 485 outbreaks of water-related disease were reported, involving 110,359 cases (Figure 2.1.1). This is an annual mean of 32.3 outbreaks per year, affecting a mean of 7,357 persons per year. The mean size of outbreaks is 228 cases, and the median size is 35 cases. Several important trends can be observed from the statistics. First, the number of reported outbreaks increased fairly steadily through 1983 with some year-to-year variation. Second, the number of reported outbreaks dropped sharply in 1984 and 1985; in 1985, the number of reported outbreaks was lower than at any point since surveillance began. Preliminary results for 1986 indicate that this recent decrease persists. The large peaks in the actual number of reported cases (Figure 2.1.2) are related to large outbreaks of undetermined etiology, the so-called acute gastrointestinal illness (AGI). In 1984 and 1985, the reported numbers of cases were the lowest since the inception of this surveillance system.

The recent and marked decrease in the reported number of outbreaks was a major focus of discussions at the recent EPA Workshop on Methods for Investigating Waterborne Disease Outbreaks. Possible explanations for this decrease include changes in surveillance itself and changes in the underlying frequency of waterborne disease outbreaks. To be reported, an outbreak must first be identified and investigated. If this decrease is the result of changes in surveillance, we must presume that waterborne disease outbreaks are still occurring in the states, but are not being recognized, investigated, or reported as frequently as they were 5 to 10 years ago. To use the "tip of the iceberg" metaphor, these explanations would suggest that the iceberg is as big as ever, but that we see less of it because it is sinking. It is important to remember, however, that substantial effort and resources have been devoted to improving the nation's water supply in recent years, including efforts to modernize treatment facilities, add filtration units, and educate the operators. If these efforts have been well-directed, we should expect the actual number of outbreaks to decrease, particularly those involving community water supplies. As a consequence, the number of reported outbreaks might also reasonably be expected to decrease. If these explanations are correct, they would suggest that we see less of the iceberg because it is in fact melting, not sinking.

Overall, the number of outbreaks related to community water supplies during the 15-year period 1971 to 1985 almost equals the number of outbreaks related to noncommunity water supplies (Table 2.1.1). Outbreaks related to community water supplies, however, affect many more people on average than do the noncommunity outbreaks. Between 1983 and 1985, the number of outbreaks related to community water supplies dropped sharply, while the number related to noncommunity water supplies remained essentially unchanged. Over the same 15-year period, the most frequent type of deficiency

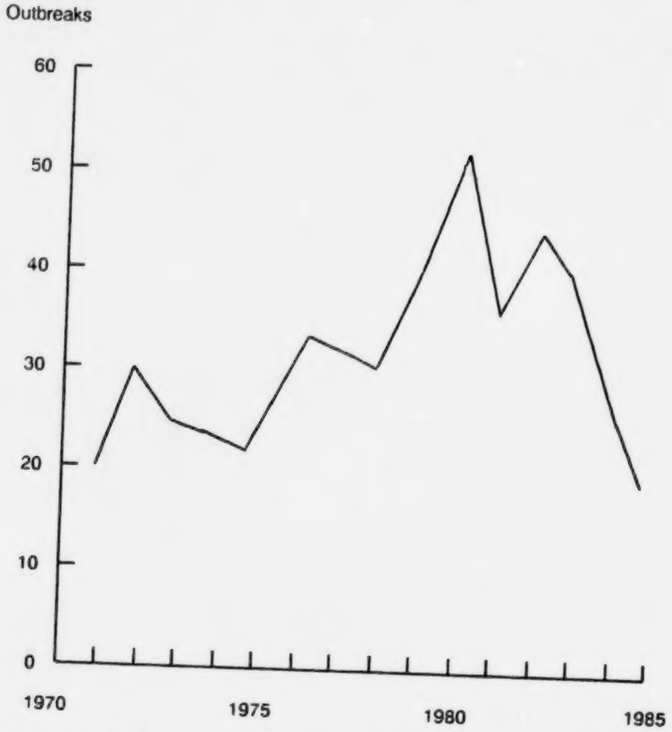


Figure 2.1.1. **Reported outbreaks of waterborne disease, by year, United States, 1971-1985.**

causing the outbreak of waterborne disease was treatment deficiency, as measured by both outbreaks and cases. Use of untreated ground water was the second most frequent cause in terms of number of outbreaks; defects in the distribution system was the second most frequent cause in terms of number of cases.

We can also describe the waterborne disease outbreaks by the etiologic cause of the outbreak. Between 1971 and 1985, 245 (50%) of the 485 outbreaks with reported cause were AGI; that is, a specific diagnosis was not reached. Ninety outbreaks (19%) were caused by parasites, 59 outbreaks (12%) by bacteria, 40 outbreaks (8%) by viruses, and 51 outbreaks (11%) by chemicals. The number of outbreaks reported each year by agents during the 15 years since surveillance began shows three distinct trends (Table 2.1.2). First, the

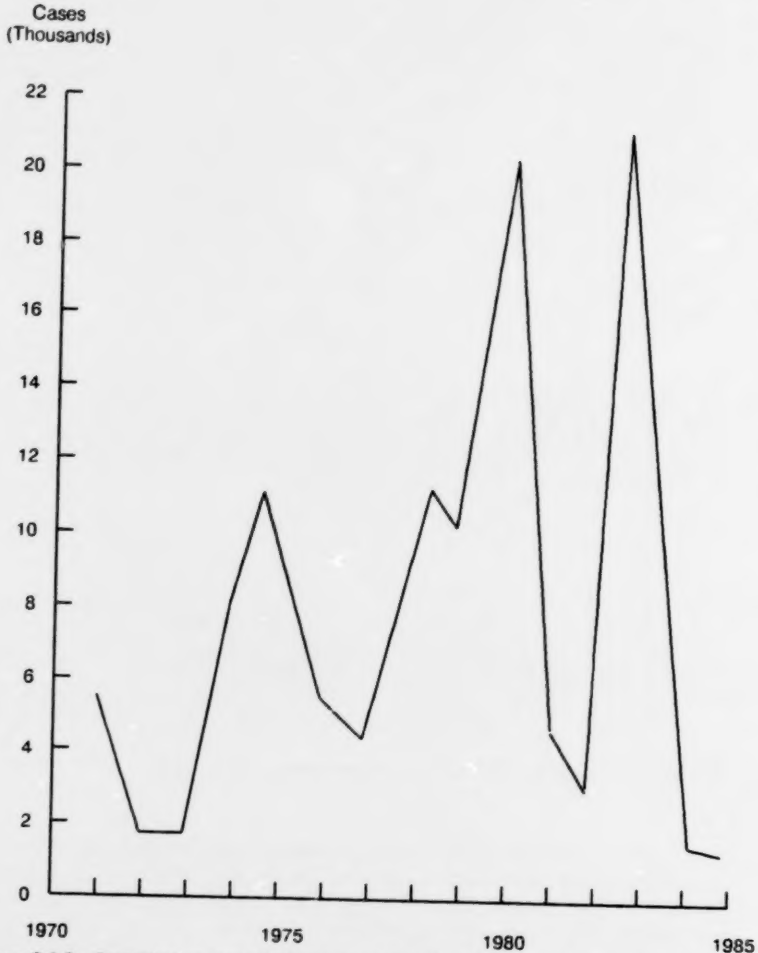


Figure 2.1.2. Reported cases of illness associated with outbreaks of waterborne disease, by year, United States, 1971-1985.

undiagnosed (AGI) outbreaks have varied in number considerably from one year to the next and account for much of the total variation in the number of outbreaks. Second, the number of reported parasitic outbreaks began to increase in the late 1970s and by 1983 accounted for a substantial fraction of all outbreaks. All but two of these parasitic disease outbreaks were caused by the parasitic pathogen *Giardia*

Table 2.1.1. **Reported Waterborne Disease Outbreaks, by Year and Type of Water Supply Systems, and Median Size of Outbreaks, United States, 1971-1985**

	Community	Noncommunity	Individual	Total	Total Cases	Median Size
1971	8	8	4	20	5,184	58
1972	9	19	2	30	1,650	26
1973	6	16	3	25	1,762	44
1974	11	9	5	25	8,356	20
1975	6	16	2	24	10,879	84
1976	9	23	3	35	5,068	30
1977	14	18	2	34	3,860	46
1978	10	19	3	32	11,435	95
1979	24	13	7	44	9,769	49
1980	26	20	7	53	20,045	40
1981	14	18	4	36	4,537	36
1982	26	15	3	44	3,588	31
1983	30	8	4	42	20,923	35
1984	12	5	8	25	1,742	20
1985	6	9	1	16	1,561	31
Total	211	216	58	485	110,359	

lamblia. Finally, in 1984 and 1985, steep declines in parasitic, viral, and undiagnosed (AGI) outbreaks have been seen.

Trends can also be seen over time in the specific causative agents. I have already commented on the rise of giardiasis outbreaks. Among the bacterial pathogens, the most common agent has been *Shigella*. In 1971 to 1973, *Shigella* represented over 70% of all bacterial disease outbreaks. The more recently discovered pathogen, *Campylobacter*, first appeared in 1977 as a waterborne pathogen and since then has accounted for a steadily increasing fraction of all bacterial waterborne disease outbreaks. In the period 1983 to 1985, *Campylobacter* accounted for more than 50% of water-related bacterial disease outbreaks. *Salmonella* is an infrequent cause of waterborne disease outbreaks; and, in particular, only two outbreaks caused by *Salmonella typhi*, the causative agent of typhoid fever, were traced to water exposures during this 15-year period. Among chemical outbreaks the most frequently reported chemical was copper sulfate (11 outbreaks), followed by hydrocarbons (7), fluoride (7), pesticide or herbicide (6), nitrates (3), and lead (3); 13 outbreaks were caused by other chemical or toxic agents. All reported viral disease outbreaks for the first 6 years of the surveillance were caused by hepatitis A virus. Since then, the Norwalk-like agents have accounted for more than 50% of viral disease outbreaks. Given the considerable technical difficulty

Table 2.1.2. Reported Waterborne Disease Outbreaks, by Type of Agent, United States, 1971-1985.

	Outbreaks					
Year	Bacterial	Parasitic	Viral	Chemical	AGI[a]	Total
1971	3	0	6	2	9	20
1972	5	4	5	3	13	30
1973	6	4	2	0	13	25
1974	5	4	0	5	11	25
1975	2	1	1	3	17	24
1976	3	3	0	3	26	35
1977	3	4	1	6	20	34
1978	7	4	3	2	16	32
1979	3	7	3	7	24	44
1980	3	8	6	7	29	53
1981	4	11	1	5	15	36
1982	3	12	7	3	19	44
1983	4	18	3	1	16	42
1984	4	7	2	3	9	25
1985	4	3	0	1	8	16
	59	90	40	51	245	485

[a] AGI = Acute gastrointestinal illness.

of diagnosing infections caused by these viral agents, they are likely to represent a larger fraction than these figures would suggest.

Thus, the general trends in pathogens reported as causes of waterborne disease outbreaks in this 15-year interval are as follows:

- The number of outbreaks caused by classic waterborne agents such as *S. typhi*, hepatitis A virus, and *Shigella* has decreased.
- Newly recognized pathogens emerging as important waterborne disease agents include *Giardia, Campylobacter*, Norwalk virus, and most recently *Cryptosporidia*.

These trends indicate the increasing importance of chlorine-resistant organisms.

To return to the issue of the recent decrease in the number of reported outbreaks: this drop occurred most sharply after 1983. This decrease probably reflects both a real decrease in the frequency with which outbreaks occur and some decrease in the proportion that are investigated. The resources available to devote to waterborne disease outbreak investigation and surveillance have decreased overall in the last 5 years. State health departments have had to cope with the tremendous challenge posed by the acquired immunodeficiency syndrome (AIDS) epidemic, often without increased resources. The resources available within EPA for advising and assisting in the evaluation of potential outbreaks of waterborne diseases decreased during this time. At CDC, the techniques of surveillance remained constant during the period 1981 to 1985. During the time of greatest decline, between 1983 and 1984, the surveillance system was maintained by the same individuals who applied equal effort in both years. Since 1985, the effort at CDC has decreased, and until 1990 a surveillance summary had not been published since that covering the year 1985.

Conclusions

In the last 2 years covered by published surveillance data, 1984 and 1985, the number of outbreaks was small. Preliminary data from 1986 through 1988 suggest this trend will continue. Further surveillance data will help to understand the causes for the decline. Several pieces of evidence, however, suggest that the decrease in reported outbreaks may be real. First, the reported outbreaks have tended to be smaller in recent years, indicating that the system is not becoming less sensitive to small outbreaks. The median size of outbreaks in the late 70s was 46 to 95, compared to the median size of 20 to 35 in the period 1983 to 1985 (Table 2.1.1). Secondly, the decrease in outbreaks has been particularly marked in community water systems where efforts to improve water supplies have been concentrated; these outbreaks are also most likely to be detected and investigated. The decrease has affected the parasitic and viral as well as undiagnosed diseases, suggesting it is not simply a decrease in specific diagnostic capacities.

A second issue before us is the effect of this decline on efforts to maintain and improve the quality of drinking water in the United States. Large waterborne disease outbreaks increase the attention given to the general problem of waterborne diseases in communities, states, and federal agencies. In the past, we have tended to depend on the acute outbreak as the primary mechanism for stimulating interest in this problem. Constant efforts are needed to continue to improve water-supply systems and to maintain the improvements in systems that have already been upgraded. We can no longer depend on the acute waterborne disease outbreak as a primary tool for educating the public or the government about the importance of these efforts. Maintaining high standards of water treatment needs to become a goal in and of itself. An appropriate analogy may be a successful vaccine against an infectious disease. When the disease is epidemic, the need for widespread use of an effective vaccine is immediately apparent to everyone. Once the vaccine has been successfully deployed and the disease becomes rare, fear of the disease itself no longer propels parents to have their children immunized. Other mechanisms must be set in place to insure high levels of vaccination in the population. The most useful measure of success of a vaccination program then becomes the proportion of the target population that is vaccinated, rather than documentation of the continued absence of the disease.

Similarly, for waterborne disease, program-based endpoints other than the number of waterborne disease outbreaks may be more useful measures of drinking water safety in the future. These endpoints may be related to the success of education programs (for instance, measures of the knowledge and practices of water treatment plant operators), to the success of efforts to upgrade treatment facilities (for instance, measures of the proportion of those drinking surface water who are served by water that is both chlorinated and filtered), or to the utility of the hazard analysis-critical control point approach to water quality testing (for instance, tests of water quality parameters during and after the critical steps of filter backwash, routine maintenance shutdown, or simulated chlorinator failure in a sample of plants).

The Future of Waterborne Disease Outbreaks and Surveillance

With some trepidation, let us gaze into the crystal ball. What do the observed trends suggest for the future of waterborne disease outbreaks? We can expect new pathogens to emerge that will evade current water treatment efforts. Chlorine-resistant organisms will represent a greater fraction of waterborne disease pathogens than in the past. Past experience shows that there is a particular risk of outbreaks occurring while a water plant is under repair or being improved. As our existing infrastructure of water treatment plants ages, it will require maintenance and replacement. It is critical that these repairs be done safely. We can expect more intense stress on available drinking water sources as the population increases, and we can also expect an increasing fraction of the population to be immunosuppressed by HIV infection, so that the presence of low numbers of pathogens in drinking water may be of more concern.

As we move into the 1990s, the federal government has several key roles to play in maintaining surveillance and expertise in the area of waterborne disease outbreaks. These include the following:

- Maintaining a national surveillance system with which to monitor new trends, assess the results of the control measures implemented, and provide feedback to the states. Maintenance of this system requires resources at the federal level that have been extremely limited in recent years.
- Maintaining and promoting expertise in epidemiologic investigation of waterborne outbreaks, including characterization of illness, technical assessment of water-treatment facilities, and use of epidemiologic methods to define the association of outbreaks with water sources. This includes providing technical support in outbreak investigations, in assessment of water-treatment facilities and water quality,

and in diagnosis of illness; and epidemiologic assistance to state health departments.

- Developing better diagnostic tools with which to assess the importance of newly described pathogens and diseases, for example, the Norwalk-like viruses and the chronic diarrhea syndrome. This requires support for the expensive laboratory procedures involved and for the search for less expensive diagnostic tools.
- Assisting in development of programmatic endpoints with which to monitor the success of drinking water purity programs.

Surveillance for Waterborne Illness and Disease Reporting: State and Local Responsibilities

by: Laurence R. Foster
State Epidemiologist
Oregon Health Division
Portland, Oregon 97201
(503) 229-5552

Introduction

The division of responsibilities between state and local public health agencies for disease surveillance and outbreak investigation differs in each state. For example, in Oregon, the local health departments are autonomous and responsible for local surveillance activities. The state health agency provides consultation, technical support, and centralized computer analysis of surveillance data. In contrast, all public health activities in New Mexico are directly supervised by the state public health agency.

No matter how the delivery of these services is organized in a state, there is a common set of basic tasks to be carried out for effective waterborne disease surveillance. For purposes of this discussion, these tasks will be considered without regard to whether the work should be done by a state or a local agency.

In order for waterborne illness surveillance to be successful, the traditional approaches must be rigorously maintained. Additional outreach, however, must be used to supplement these traditional approaches.

The first purpose of surveillance for waterborne disease is immediate prevention of further illness once an outbreak has started. The surveillance system should be sensitive enough to detect outbreaks early in their course. Such detection should trigger epidemiological and environmental investigation of a suspected outbreak. If such investigation determines drinking water to be the source of illness in an outbreak, control measures can be rapidly initiated to prevent further illnesses. Such control measures include short- term solutions such as boil water notices, increased disinfection, or recommending bottled water or alternative water sources. They also include long-term measures such as disconnecting a cross connection, improving water treatment practices, and upgrading water treatment facilities.

Surveillance for waterborne disease includes three major categories of tasks: case identification, case evaluation, and outbreak investigation. Case identification involves obtaining reports of cases of illness. Case evaluation means determining from individual cases whether a group of illnesses may potentially be from a common source and whether drinking water is a potential source. The outbreak investigation then determines whether there is an outbreak, and, if so, its probable source.

Case Identification

The traditional approach to case identification is for the state public health agency to declare that certain diseases are reportable by law or administrative rule. Physicians, and often other health care providers, are obligated to report individuals with certain diagnoses. Examples of potentially waterborne diseases that are reportable in Oregon include giardiasis, hepatitis A, campylobacteriosis, yersiniosis, shigellosis, and salmonellosis. In Oregon, an "extraordinary occurrence of illness" is also reportable. This means that when a health care provider recognizes an unusual number of cases, or a clustering of cases, of an illness that is not specifically reportable, he or she must report that fact to the local health department. This requirement is intended to promote identifications of outbreaks caused by agents that are not otherwise reportable, such as Norwalk agent and toxic substances.

Generally a case report must include the patient's name and locating information, the doctor's name and address, and the specific diagnosis. Suspected cases of illness, in addition to confirmed cases, have been made reportable in Oregon to improve the sensitivity and timeliness of the surveillance system. Reports must generally be made within one working day of the time of exposure, but extraordinary occurrence of illness is to be reported immediately, day or night.

Oregon also requires that clinical laboratories report the identification of certain disease-causing agents or markers for infection by those agents. For example, positive cultures for *Campylobacter jejuni* and *Shigella* species, positive stool examination for *Giardia lamblia* and *Entamoeboa histolytica*, and a positive IgM antihepatitis A virus antibody test are reportable. The Oregon Health Division has found that this greatly enhances the completeness of reporting.

To further enhance reporting, we have discovered that we must go beyond the traditional approach to surveillance described above. This necessity has been demonstrated by the fact that for most of Oregon's documented waterborne disease outbreaks, the initial case report came from a private citizen. In fact, all of Oregon's larger waterborne outbreaks have come to our attention that way. This may be because Oregon's larger waterborne disease outbreaks have occurred in communities heavily frequented by tourists. Initial reports from these outbreaks have come from visitors to the outbreak communities who have become ill after returning to their homes.

These experiences tell us that public health agencies should seek reports of suspected waterborne illness from the general public. Although there is no simple formula for doing this successfully, it helps to maintain a high public profile for epidemiology and disease control activities generally. Significant events related to outbreaks and disease control should be actively reported to the news media, within the limits of confidentiality and professional propriety, of course. Epidemiology staff should always be willing to help reporters get the information they need, again within the limits of confidentiality and propriety. The epidemiology unit should publish a high profile newsletter directed toward health care providers, but also sent to the news media. This newsletter should be clinically relevant, concise, and informative, and its topics should be timely relative to news releases. All of these efforts should promote an image of the health agency as concerned and responsive, thereby encouraging citizens to report to the agency when they develop an illness and suspect it to be from drinking water.

Another useful outreach focus to supplement traditional approaches to surveillance is the water system operator. These officials regularly receive complaints from their customers, and those complaints sometimes are about illnesses suspected to have been caused by the drinking water. In Oregon, the Water Quality Program has developed a standardized complaint form to help system operators collect systematic information from complainants. When an illness is part of the complaint, the operator can then turn the information over to the public health agency for further investigation.

The surveillance component for case identification should, therefore, include requirements for reporting of specific diseases and extraordinary occurrences of illness by health care providers, encouraging reporting by private citizens, and developing specific mechanisms to improve reporting of customer complaints by water system operators.

Case Investigation

When an individual case of a reportable disease is reported, health agency staff carry out an investigation of that case to identify the possible source of infection, as well as to prevent further spread. For example, when a case of hepatitis A is reported, the health department epidemiologist tries to determine the probable source, such as a family member with recent hepatitis, day care, foreign travel, a restaurant, or drinking water potentially contaminated with human feces. Possible sources to be sought by interviewing a giardiasis case would include water from a mountain stream, a community drinking water system, or a day care.

The epidemiologist usually cannot draw a conclusion about a common source, such as a community water supply, on the basis of a single case investigation. However, the investigator may suspect community drinking water to be the source of infection for an individual, if an alternative source of infection cannot be identified, and if the individual drank water from a system with inadequate treatment. This suspicion may seem to be confirmed particularly if the relative timing between an individual's drinking of the water and the onset of illness fits the known incubation period for the particular disease.

The epidemiologist, however, usually suspects a community drinking water supply to be the source of infection only when a cluster of cases is identified among people who drank the water and who have similar dates of onset, and no alternative sources of infection are identified. Detecting such clusters of cases requires alertness on the part of the investigator to recognize when an unusual number of cases of a particular illness is occurring. This recognition requires a standardized approach to individual case investigation, as well as thorough training and a high degree of motivation.

Computer review of the number of cases reported each week is also useful in detecting unusual clusters of cases in jurisdictions with sufficient population to make computer review more efficient than manual review. In Oregon, the number of cases of each reportable disease reported each week is tabulated by computer. A computer program has been developed to identify those counties that have experienced a number of cases for a particular disease that exceeds the 99 percent confidence interval for number of cases expected for that week. This 99 percent confidence interval is based upon the weekly average over an 8-week time period during the same season of the previous year.

One way to screen for a possible waterborne outbreak is to tabulate reported cases within various drinking water systems to determine if the expected number of cases is exceeded. However, in Oregon, we have found that routinely tabulating reported cases of illness by drinking water system or source was not feasible due to the many different sources of water in the state, the use of more than one water source by most individuals, and the lack of a standardized nomenclature for recording an individual's drinking water sources.

The cost effectiveness of investigating individual cases of reportable illness should be evaluated for each disease and in each state. Although such cost effectiveness has not been rigorously studied in Oregon, experience has shown that routine individual case investigation may not be worth the effort for some diseases.

In 1981, campylobacteriosis and giardiasis were made reportable in Oregon. Local health departments were required to perform individual case investigations and to complete standard interview forms for every case. The resulting workload was immense. The two diseases quickly became the most commonly reported diseases in the state, second only to gonorrhea. Therefore, the number of required individual investigations was large. The extensive effort required was not productive; only two small community waterborne disease outbreaks were identified with 3 years of such effort. These outbreaks could have been identified simply by observing that an unusual number of cases were occurring in the affected communities. The individual case investigations did not enhance the sensitivity or timeliness of simple case count tabulation for identifying the occurrence of these outbreaks.

Because of this finding, individual cases of campylobacteriosis and giardiasis are no longer investigated in Oregon unless the number of cases in a county exceeds the threshold limit. In other words, individual case investigations are initiated only when an unusual number of reported cases suggest a common source outbreak may be occurring.

Outbreak Investigation

Once the epidemiologist suspects, on the basis of individual case investigations, that a waterborne outbreak may be occurring, a full-fledged outbreak investigation should be started immediately. Such an investigation should first seek to determine whether drinking water really is the source of the outbreak. If it is, the second stage of this investigation is to help end the outbreak. Outbreak investigations are discussed in more detail in another chapter.

Conclusion

Surveillance for waterborne disease at the state and local level must include case identification, individual case investigation, and the carrying out of an outbreak investigation. In addition, public health agencies should continually evaluate the effectiveness and efficiency of their surveillance systems in order to seek improvements.

Although public health agencies should emphasize the value of an effective surveillance system and maintain constant vigilance for waterborne disease, they must not promote the mentality that "everything is okay" if no outbreaks are identified. Our real emphasis must be on prevention by improving currently inadequate drinking water systems and by attending to the proper long-term maintenance and operation of those systems that are currently good ones.

III. Investigation of Waterborne Disease Outbreaks

Differences Between Outbreak Investigation and Research Epidemiology

by: Neal D. Traven
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh
Pittsburgh, Pennsylvania 15261
(412) 624-0097

Introduction

Epidemiology, in one form or another, has been practiced for thousands of years. For example, in the treatise *Airs, Waters, Places*, Hippocrates recognizes the importance of environmental changes and behavioral patterns in the study of human disease patterns (1). The *Old Testament* book of *Daniel* (Chapter 1, Verses 1-15) recounts the tale of an experiment comparing two dietary regimens; in the end, Daniel's diet is found to be more healthful than the king's (2).

The origins of modern epidemiology can be traced from John Graunt's 1662 studies of mortality patterns in London, through the 1747 demonstration by James Lind of the efficacy of citrus fruits in combatting scurvy, to the appointment of William Farr as the first Registrar-General of England's General Registry Office in 1839 (3). Farr and his colleagues founded the London Epidemiological Society in 1850.

Among those colleagues was the eminent physician John Snow, already well known in London for having administered chloroform to Queen Victoria during childbirth. Snow investigated a series of cholera outbreaks in London during the period 1848-1854 (4).

At that time, two companies supplied water to most of the homes in a section of London south of the Thames River; their systems were so intertwined that adjoining buildings might be served by different suppliers. Though both companies took their water from the sewage-laden Thames, one had recently moved its intake to a less contaminated section of the river. By comparing the addresses on water bills to those on cholera death certificates (provided by Farr's General Registry Office), Snow demonstrated a nearly ninefold increase in cholera mortality in homes supplied by one of the companies (see Table 3.1.1). Snow's conclusion that something in Southwark and Vauxhall's water supply caused cholera was so

Preceding page blank

persuasive that all of London's suppliers were required to filter their water by 1857, just 2 years after he published his findings.

Table 3.1.1. Cholera Deaths per 10,000 Houses by Water Supply, London, 1854*

Water Supply	Number of Houses	Deaths from Cholera	Deaths per 10,000 Houses
Southwark and Vauxhall Company	40,046	1,263	315
Lambeth Company	26,107	98	37
Rest of London	256,423	1,422	59

*Adapted from (4).

It should be noted that Snow's work, as well as that of Semmelweiss on puerperal fever, Budd on typhoid fever, and Panum on measles, predated the germ theory promulgated by Pasteur and others. Indeed, the cholera vibrio was not identified until 1883, by Robert Koch.

Outbreak Investigation or Epidemiology?

The late 19th century saw an explosion of public health action against many of the diseases that had plagued mankind throughout history. In rapid succession, the infective agents and modes of transmission of malaria, tuberculosis, diphtheria, scarlet fever, typhus, tetanus, rabies, smallpox, and many others were uncovered. In many cases, researchers devised vaccines capable of preventing the occurrence of the diseases; for other infectious diseases, improvements in medical care and the advent of antibiotics could cure those who contracted them.

Underlying these monumental achievements was a newly developed fundamental concept of the mechanisms of infectious diseases. Koch's postulates (Table 3.1.2) were formulated by the German pathologist-microbiologist around 1890. These axioms define a strong causal relationship between microorganisms and specific diseases (5). In the century since the advent of microbiology, it has been recognized that Koch's postulates overstate the causal association between microorganisms and diseases; later research in immunology, microbiology, and related fields has unearthed the complexities of infectious diseases. The eminent American infectious disease epidemiologist Alfred Evans has since developed an extended set of postulates based on Koch's (Table 3.1.3) (6).

Mankind's victory over the plagues of yesteryear, capped by the total eradication of smallpox from the planet, means that we are now only rarely subjected to epidemics of infectious diseases. In

Table 3.1.2. Koch's Postulates

- The agent must be shown to be present in every case of the disease by isolation in pure culture.
- The agent must not be found in cases of other disease.
- Once isolated, the agent must be capable of reproducing the disease in experimental animals.
- The agent must be recovered from the experimental disease produced.

Table 3.1.3. Evans' Postulates*

- Prevalence of the disease should be significantly higher in those exposed to the hypothesized cause than in controls not so exposed.
- Exposure to the hypothesized cause should be more frequent among those with the disease than in controls without the disease - when all other risk factors are held constant.
- Incidence of the disease should be significantly higher in those exposed to the hypothesized cause than in those not so exposed, as shown by prospective studies.
- The disease should follow exposure to the hypothesized causative agent with a distribution of incubation periods on a a bell-shaped curve.
- A spectrum of host responses should follow exposure to the hypothesized agent along a logical biological gradient from mild to severe.
- A measurable host response following exposure to the hypothesized cause should have a high probability of appearing in those lacking this before exposure, or should increase in magnitude if present before exposure. This response pattern should occur infrequently in those not so exposed.
- Experimental reproduction of the disease should occur more frequently in animals or humans appropriately exposed to the hypothesized cause than in those not so exposed; this exposure may be deliberate in volunteers, experimentally induced in the laboratory, or may represent a regulation of natural exposure.
- Elimination or modification of the hypothesized cause should decrease the incidence of the disease (i.e., attenuation of a virus, removal of tar from cigarettes).
- Prevention or modification of the host's response on exposure to the hypothesized cause should decrease or eliminate the disease (i.e., immunization, drugs to lower cholesterol, specific lymphocyte transfer factor in cancer).
- All of the relationships and findings should make biological and epidemiologic sense.

*Adapted from (6).

contemporary industrial and post-industrial societies, the few outbreaks of acute infectious disease are investigated rapidly.

Waterborne disease outbreaks are usually found to result from a breakdown in the mechanical systems that ordinarily protect us from waterborne pathogens (e.g., drinking water or sewage treatment plants). These breakdowns are nearly always amenable to engineering solutions. Outbreak investigation can be likened to "firefighting" - when an outbreak flares, investigators rush to the scene, assess the damage, find its cause, correct the problem, and return the system to its normal state.

On the other hand, today's plagues are largely chronic degenerative conditions like heart disease, cancer, pulmonary disease, arthritis, and many others. With these diseases, strong relationships with specific causative agents are observed very rarely. Chronic diseases are associated with complex interactions within the "epidemiologic triad" of host, agent, and environment. For example, insulin-dependent diabetes mellitus may be triggered by a disordered host response to viral infections in persons with a genetic predisposition to the disease. The infection may lead to diabetes, however, only if the individual is in a particular susceptible age range (generally, under age 20). But even in the presence of susceptible age, viral infection, and genetic predisposition, there is no guarantee that diabetes will result; nor does Type I diabetes occur only in persons with such a profile. In addition, the risk factors most strongly associated with contracting a disease may differ greatly from those related to its progression and outcome. Finally, a disease may itself be a risk factor for other diseases; for example, Type I diabetes mellitus greatly increases the risk of coronary heart disease in young men.

Even where a disease-pathogen relationship exists (e.g., AIDS and HIV), then, epidemiologic research no longer studies disease causation; these multifactorial diseases do not have single causes. Instead, epidemiologists search for risk factors of disease. The presence of risk factors, such as those described above for diabetes mellitus, increases the probability of disease, but does not cause it.

Contemporary epidemiology is a probabilistic endeavor in that it often boils down to a search for personal behaviors, genetic propensities, and/or environmental exposures that may affect the likelihood of disease occurrence or progression. These risk factors may have occurred in the remote past. Epidemiologists seek "associations" between disease states and risk factors, taking into account the possible independent and interactive effects of other potential risk factors. Rather than Koch's deterministic postulates, contemporary epidemiologists apply criteria such as those put forward by Evans or Bradford Hill (7) to assess evidence about association between diseases and potential risk factors (see Table 3.1.4).

It should be noted that these postulated criteria for the conduct of epidemiologic research overlap significantly. For example, it is difficult to differentiate between Bradford Hill's criteria of biological plausibility and coherence. Taken as broad guidelines, however, they provide a valuable underpinning for epidemiologic researchers. The concepts of specificity of effect, dose-response relationship,

Table 3.1. 4. Assessment of Epidemiologic Evidence About Associations with Potential Risk Factors*

- *Consistency* – the association is consistent if the results are replicated when studied in different settings and by different methods.
- *Strength* – an expression of the disparity between the frequency with which a factor is found in the disease and the frequency with which it occurs in the absence of the disease.
- *Specificity* – established with the limitation of the association to a single putative cause and single effect.
- *Dose-response* relationship – established when an increased risk or severity in disease occurs with an increased quantity ("dose") or duration of exposure to a factor.
- *Temporality* – the exposure to a putative cause always precedes, never follows, the outcome.
- *Biological plausibility* – it is desirable that the association agree with current understanding of the response of cells, tissues, organs, and systems to stimuli.
- *Coherence* – associations should not conflict with the generally known facts of the natural history and biology of disease.
- *Experiment* – it may be possible to appeal to experimental, or quasi-experimental evidence, e.g., an observed association leads to some preventive action.

*Adapted from (7).

temporality, and biological plausibility remain essential to investigators of waterborne diseases.

Epidemiologic Studies – Design and Evaluation

Study Designs

The two primary epidemiologic study designs are case-control and cohort studies. In the former, subjects are grouped according to their disease status; their prior behaviors or exposures are investigated retrospectively. The latter type of study follows subjects who are initially free of the disease, determines their exposure or behavior, and examines their disease status after a (usually lengthy) period of time.

The history of case-control studies has been traced by Lilienfeld and Lilienfeld (3) to the French physician-mathematician P.C.A. Louis and his 1825 paper on tuberculosis. The first modern case-control study, on breast cancer, was published by J.E. Lane-Claypon in 1926. In the contemporary era, since World War II, case-control studies have become increasingly prevalent in the epidemiologic literature. In recent years, much methodologic research in the field has been concentrated on improving the sophistication of case-control analysis.

Each of the major types of epidemiologic study has its advantages and its disadvantages. Cohort studies are extremely expensive and time consuming, since the disease of interest may take decades to develop; for example, the Framingham Heart Study has been underway for over 40 years. They also require a large number of subjects, since even an extremely common disease may affect only 1 in 10 or 20 participants. The extended follow-up period is likely to result in attrition problems, and changes in standard medical practice may invalidate study criteria and methods (8). Conversely, only in a prospective cohort study can one determine incidence rates and relative risks. Also, the temporal sequence of risk factor and disease can be demonstrated convincingly only with the cohort design.

Case-control studies require many fewer subjects than cohort studies. With rare diseases (and fortunately, most diseases do occur infrequently), the case-control study is the only practical design, though it can be applied to the analysis of any disease. Case-control studies can usually be completed more quickly than cohort studies, taking years rather than decades. Due to their retrospective nature, however, case-control studies are particularly susceptible to biased recall of events in the remote past and to incomplete information. Statistically, they yield only odds ratios, though this quantity is often an excellent estimate of the true incidence rate (9). The primary difficulty in designing a case-control study, however, is the selection of appropriate control subjects. Often, controls are matched with cases on a number of variables in order to increase their comparability. Many epidemiologic studies have been subjected to criticism regarding the appropriateness of the selected control groups and potential problems in generalizing study results.

Evaluation of Epidemiologic Data

Such basic tools for evaluating epidemiologic results as incidence rate, rate differences, relative risk, and odds ratio are described in another paper in the proceedings (10). As noted there, the odds ratio has become the measure of choice used by epidemiologists to investigate associations between risk factors and diseases. In any epidemiologic study, it is always necessary to address potential biases in sample selection, questionnaire design, recall by subjects, and a host of other sources; these subjects are dealt with in a paper by Richard Vogt, also in these proceedings.

Another issue to be faced in the analysis of epidemiologic data is confounding by other variables. A confounder is an extraneous variable which can account for some portion of the apparent effect of the risk factor of interest. As described by Rothman (11), a confounder is itself a disease risk factor which is associated with the study variable but is not a consequence of it. For example, in a study of the effects of alcohol consumption on throat cancer, cigarette smoking could confound the relationship under investigation; smoking is also a risk factor for throat cancer and is itself associated with alcohol use.

The effects of confounding variables on the association of interest can be negative or positive, large or small. In other words, a confounder may mask a real association (yielding a spuriously nonsignificant odds ratio), or it may produce a falsely significant relationship. The epidemiologist, then, must always be aware of putative risk factors other than those in which he or she is interested, and should ensure that questionnaires are written to address those issues.

The effects of potential confounders can be removed or controlled by choosing the appropriate study design. In a matched design, control subjects are chosen with foreknowledge of their status on selected factors. By ensuring that cases and controls do not differ with respect to those factors, this design eliminates the potential confounding effect of the matching variables. The disadvantages of this approach are that the matching variables cannot be used in the analysis and that it may be difficult to find close matches (particularly when matching simultaneously on several variables) unless the pool of potential control subjects is very large.

An alternative strategy is to use epidemiologic methods to evaluate and statistically control for confounding. The two techniques most widely used to control for confounding are stratification and multivariate analysis.

In stratified analysis, the basic fourfold table calculation of the odds ratio is carried out for each of several levels or strata of the potential confounder. For instance, one might determine the odds ratio within each of three or four age strata. These separate stratum-specific odds ratios can be combined into a single summary odds ratio (controlled for levels of the potential confounder) using the procedure outlined by Mantel and Haenszel in their seminal 1959 paper (12). Mantel-Haenszel stratified analysis also provides a one degree-of-freedom summary chi-square for the overall association between the study variable and the disease.

The Mantel-Haenszel technique can be extended to account for several potential confounders. But unless the original sample is very large, one quickly finds many cells with very small numbers of subjects. In addition, the Mantel-Haenszel technique may require the researcher to impose artificial stratification or arbitrarily chosen cutpoints on some potential confounders.

The other method to control for confounding in epidemiologic analysis is the multivariate technique known as logistic regression. In this procedure, the data are fitted to the model:

$$P(y|x) = \frac{1}{1 + \exp[-\alpha - \Sigma(\beta_i x_i)]}$$

where $P(y|x)$ is the probability of disease and the β_is are regression coefficients for the predictor variables (the x_is), among which is the study risk factor. In this model, the predictor variables may be discrete or continuous. Under appropriate conditions, logistic regression coefficients are algebraically identical with the logarithm of the odds ratio (13), so the logistic model is used to determine an odds ratio for the variable of interest while simultaneously controlling for many potential confounding factors.

In most cases, results from the Mantel-Haenszel procedure and the multiple logistic model are quite similar. In their recent textbook on epidemiologic statistics, Kahn and Sempos (13) illustrate the near-equivalence of these methods applied to Framingham data.

Summary

Much of the early history of epidemiology, such as Snow's work on cholera, revolved around investigation of outbreaks of acute infectious diseases. Investigation of waterborne disease outbreaks still owes much to these methodologies developed in the 19th and early 20th centuries. Improvements in sanitation and drinking water purification, advances in microbiologic identification of pathogens, and the wide availability of vaccines, antibiotics, and medical care have vastly decreased the number and scope of such outbreaks in industrial and postindustrial societies. Since World War II, then, the research focus of epidemiologists has turned increasingly to investigation of such chronic conditions as heart disease, cancer, diabetes mellitus, and a host of others.

In a research environment where Koch's postulates are of diminished usefulness, epidemiologic study designs and analytic methodologies have been developed to examine such multifactorial disorders. Instead of "causes," epidemiologists now search for "risk factors" for diseases. Much attention is paid to associations between diseases, the influence of health behaviors, environmental and occupational exposures, genetic predisposition, and many other confounding or interacting factors. Selection of appropriate study subjects is of overwhelming importance in such research. In addition, the purview of epidemiologic methodology has now expanded to encompass such fields as controlled clinical trials and evaluation of health programs.

Research Opportunities

As indicated earlier, investigation of waterborne disease outbreaks is largely a matter of identifying the pathogen, determining where an equipment failure has permitted it to enter the water supply, and repairing the damage. The sophisticated methodologies of contemporary epidemiology have not supplanted the traditional techniques deriving from the days of William Farr and John Snow in such short-term problem resolution.

Nothing said in this chapter should be taken as a "put-down" of the methods used in the epidemiologic investigation of waterborne disease outbreaks. In a recent paper (14), Kuller elucidates the relationship between chronic and infectious disease epidemiology, placing special emphasis on the similarities between them. As he points out, all epidemiologic studies strive to identify the determinants of diseases in order to decrease the societal burden of morbidity and mortality they carry. The traditional goals of infectious disease epidemiology - recognition of an agent, incubation period, mode of transmission, and so forth - have proved extremely difficult for chronic disease investigators to achieve. Because the incubation period of a chronic disease is so long, and the spectrum of disease is so broad, these traditional approaches have (perhaps unfairly) been superseded by complex multivariate statistical techniques and the concept of multifactorial etiology of disease.

The methodologies of chronic disease epidemiology certainly have a place when considering the possible long-term effects of drinking water consumption. Craun (15) has reviewed the evidence that chlorination by-products, particularly trihalomethanes, might be a risk factor for gastrointestinal and genitourinary cancers, as well as for cardiovascular diseases. In addition, some studies have found associations between hard water and decreased risk of cardiovascular diseases, and others have examined the possible effects of ingestion of sodium or other salts on blood pressure and other physiologic functions.

The seroepidemiology approach developed by Albert Evans may be a valuable avenue for collaboration between infectious disease and chronic disease epidemiologists. Evans advocates using serum banks to investigate disease risk among persons seronegative or seropositive to a particular organism, or to evaluate potential associations between seroconversion and subsequent risk of disease (16). As new serologic tests are developed, one might, for example, be able to estimate baseline population exposure to waterborne pathogens. As new pathogens are characterized, stored sera could be examined for presence of antibodies to the organism. This method has already been used to characterize a "Pontiac fever" outbreak (which occurred before isolation of *Legionella* in 1976) as due to that organism, and to trace the introduction of the HIV virus into the United States.

References

1. Hippocrates. The Genuine Works of Hippocrates. Translated from the Greek by Francis Adams. Williams and Wilkens, Baltimore, Maryland, 1939. p. 19.

2. Slotki, J.J. Daniel, Ezra, Nehemiah. Hebrew Text and English Translation with Introductions and Commentary. The Soncino Press, London, England, 1951. p. 3.

3. Lilienfeld, A.M. and Lilienfeld, D.E. Foundations of Epidemiology. Second edition. Oxford University Press, New York, New York, 1980.

4. Snow, J. On the Mode of Communication of Cholera. Second edition. Churchill, London, England, 1855. Reprinted as Snow on Cholera. The Commonwealth Fund, New York, New York, 1936.

5. Last, J.M. A Dictionary of Epidemiology. Second edition. Oxford University Press, New York, New York, 1988.

6. Evans, A.S. Causation and disease: The Henle-Koch postulates revisited. *Yale J. Biol. Med.* 49: 175, 1976.

7. Bradford Hill, A. The environment and disease: Association or causation. *Proc. Roy. Soc. Med.* 58: 295, 1965.

8. Mausner, J.S. and Bahn, A.K. Epidemiology: An Introductory Text. W.B. Saunders, Philadelphia, Pennsylvania, 1974.

9. Schlesselman, J.J. Case-Control Studies: Design, Conduct, Analysis. Oxford University Press, New York, New York, 1982.

10. Traven, N.D. Data analysis: Estimating risk. *In:* G.F. Craun (ed.) Methods for Investigation of Waterborne Disease Outbreaks, Report No. EPA/600/1-90/005a, U.S. Environmental Protection Agency, Cincinnati, OH, 1990, pp 65-74..

11. Rothman, K.J. Modern Epidemiology. Little, Brown and Company, Boston, Massachusetts, 1986.

12. Mantel, N. and Haenszel, W. Statistical aspects of the analysis of data from retrospective studies of disease. *J. Natl. Cancer Inst.* 22: 719, 1959.

13. Kahn, H.A. and Sempos, C.T. Statistical Methods in Epidemiology. Oxford University Press, New York, New York, 1989.

14. Kuller, L.H. Relationship between acute and chronic disease epidemiology. *Yale J. Biol. Med.* 60: 363, 1987.

15. Craun, G.F. Surface water supplies and health. *J. AWWA.* 80(2): 40, 1988.

16. Evans, A.S., Kirchhoff, L.V., Pannuti, C.S., and Carvalho, R.P.S. A case-control study of Hodgkin's disease in Brazil. II. Seroepidemiologic studies in cases and family members. *Am. J. Epidemiol.* 112: 609, 1980.

Epidemiologic Principles for the Study of Waterborne Outbreaks

by:

Richard L. Vogt, M.D.
Vermont State Epidemiologist
Vermont Department of Health
Burlington, Vermont 05401
(803) 863-7240

Introduction

There are at least three goals for investigating an outbreak potentially caused by a waterborne agent. The first goal of any waterborne disease investigation is to protect the health of people at risk by stopping the waterborne transmission of the agent. Early in the investigation, this may require identifying another water source or recommending boiling water that will be consumed if waterborne disease is strongly suspected. The next goal is to determine the cause of the outbreak in order to correct any problems that may be contributing to contamination of the water system. This should provide longer term protection against waterborne illnesses for the community. Finally, a third goal is to learn about new aspects of waterborne disease that may apply to other communities (1).

Outbreak Determination

Waterborne illness comes to the attention of a public health agency through several mechanisms. Information may be obtained from a variety of sources including health care providers, water supply operators, town or local health officials, health department surveillance reports, laboratory reports, the general public, and the news media. Reports can vary from a single case of cholera, to a report that a whole town appears to be ill. Frequently, the earliest challenge is to determine if an outbreak has occurred.

After a health department receives a report of an unusual occurrence of disease that may be a waterborne outbreak, an investigation is undertaken. The investigation may or may not be carried out to completion, depending upon what is determined in the early stages of the study. First, it is important to verify an outbreak and determine the diagnosis of the ill persons, if possible. Next, health officials need to establish the extent of the problem by searching reliable sources of data, including records in physicians' offices, emergency rooms, and laboratories.

If a likely outbreak is identified, investigators must gather demographic information and attempt to summarize the reports of illness by three variables: person, place, and time. This characterization will assist in testing scientific hypotheses about the cause and possibly the effect of illness.

In order to organize the illness reports by person, place, and time, investigators need to develop a case definition. The case definition is determined by first identifying the predominant signs and symptoms of ill persons in the outbreak and developing inclusion criteria. These criteria serve to identify persons with illness that is characteristic of the outbreak and exclude those who are well or ill with different symptoms. Developing a case definition will enable the investigator to specifically categorize those surveyed as "cases" or "noncases." Investigators can then graph the numbers of cases of illness over time to develop an epidemic curve.

The known incubation periods for commonly identified waterborne pathogens are shown in Figure 3.2.1. Figure 3.2.2 shows an example of a point source outbreak; that is, an outbreak caused by a single source of contamination occurring over a brief period of time. Using an epidemic curve that shows a pattern typical of a point source outbreak, one can determine either the incubation period of the agent or the likely date of contamination if the other variable is known. This is because the interval between the time of contamination and the peak of the outbreak will approximate the incubation period of the illness.

Testing a Waterborne Hypothesis

If the early steps of the investigation suggest that waterborne transmission is likely, it is important to clearly establish drinking water as the source responsible for the illness. Agents that can cause waterborne illness may be transmitted through food and person-to-person contact, as well.

Contaminated water can be ingested through many possible vehicles, simultaneously. As Table 3.2.1 demonstrates, there is no single source for infection from contaminated water. Combining all exposures to unheated tap water may combine unequal risks and will reduce the ability to determine the likely vehicle of infection.

To specifically test a hypothesis that water has caused illness, there are several study designs that can assist the investigator. If the outbreak was identified through health department reports of reportable diseases, one can use the information to determine if there is an excess of disease within a specific water delivery area. This analysis can be useful if an outbreak seems to be continuing over a

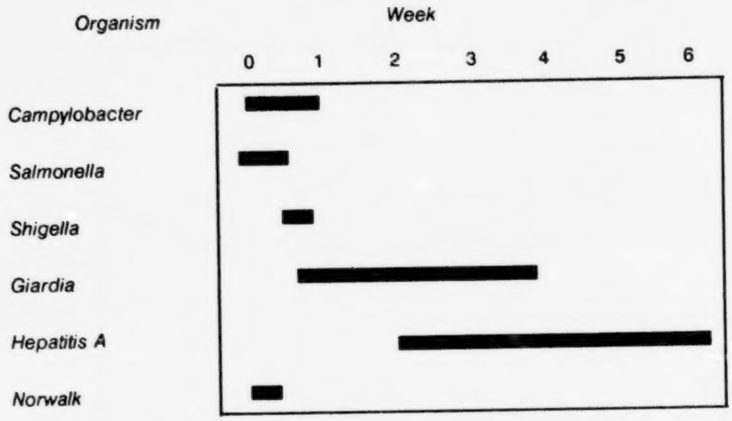


Figure 3.2.1. Incubation periods of waterborne microbial agents.

long period of time. If during the process of an outbreak investigation, the causative organism is identified, it may be helpful to analyze disease surveillance reports by geographic area. Information on previous illness can serve as a comparison for the current case reports or can tell the investigator whether the illness is a new or old problem in that particular geographic area. Unfortunately, it may take some time for reports to be received by the health department. As a result, health departments may not be able to create timely information through this data source to assist in the investigation of possible waterborne illness.

To test the hypothesis that water may have caused illness, investigators may conduct one of two more labor-intensive studies: the follow-up or case-control study. A follow-up study first identifies a community that has both individuals who are exposed and unexposed to the probable vehicle of infection. Illness is then ascertained for each of the exposed and unexposed groups of individuals and rates of illness are calculated. An example of a group that could be used for a follow-up study would be church members who drank water from a church well that was contaminated with sewage. First, it is determined which individuals did and did not drink from the church water fountain and, subsequently, which individuals developed illness.

Investigators may also use a case-control study design to test the waterborne illness hypothesis. Cases are persons who have already been identified by the "case definition" as having illness that may have been caused by contaminated water. Controls are the noncases who are

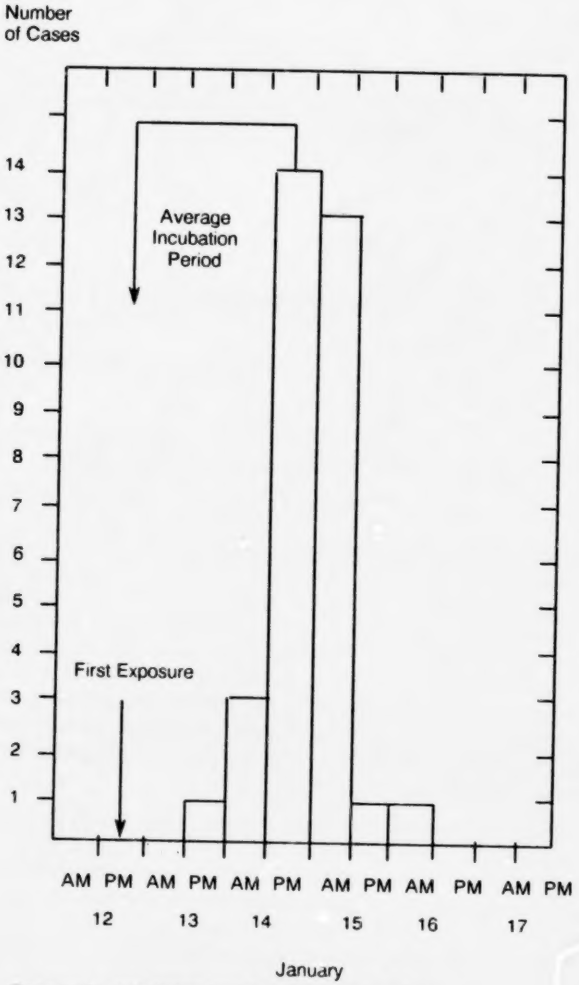


Figure 3.2.2. Cases of gastrointestinal illness by date of onset.

in the same catchment area as cases. Example of cases could be persons diagnosed with shigellosis from one doctor's office; controls could be a sample of noncases taken from a list of all patients in the same doctor's office. After cases and controls are selected for study, their exposure to drinking water is determined. For example, a history of attendance at

Table 3.2.1. Possible Vehicles for Waterborne Illness

- Unheated Tap Water
  - Drinking Water
  - Water Used for Brushing Teeth
- Unheated Tap Water Beverages
  - Orange Juice
  - Instant Iced Tea
  - Kool-Aid
  - Lemonade
  - Milk Made with Powdered Milk
  - Ice

one church would be obtained and, even more specifically, consumption of water at the church water fountain would be determined for each patient. The challenge with the case-control method is to choose controls that have an equal likelihood of becoming cases except for exposure to the agent in question. If cases were identified through physicians' logs, controls should probably be selected through this mechanism as well.

A questionnaire is administered to a survey population with both study designs to obtain information on exposures and illness. With the follow-up study design, random-digit telephone or door-to-door household surveys are two ways to administer the questionnaire to test a hypothesis that water has caused illness. If people in either large or small groups, such as persons in camps, those attending a family gathering, or employees in an industry, can be identified as having been exposed to contaminated water, the entire group can be studied. If sufficient numbers of individuals did and did not consume water, it can be determined whether contaminated water caused illness. The ability to statistically determine whether water caused illness is markedly diminished if the vast majority of the group was exposed to the vehicle. In addition, if only a small group or sample of a large group is studied, it is important that the group is representative of others who may be exposed to the contaminated water. Otherwise, it will be difficult to generalize the results of the epidemiologic investigation beyond the sample.

Using the follow-up study design, Figure 3.2.3 shows how illness rates, also called attack rates, are calculated for those who are and are not exposed to contaminated water. If the rates are higher for those who are exposed compared to those who are unexposed, water may have caused illness. One further calculation can be made with the comparison of illness rates, the calculation of the relative risk. The relative risk is defined as the illness rate of those exposed divided by the illness rate of those unexposed and indicates the magnitude of risk for waterborne exposure (Figure 3.2.4). The resultant expression is a ratio that compares the relative likelihood of becoming ill after exposure to the likelihood of illness for those unexposed. Illness rates and relative risks cannot be calculated in the case-control study. An estimate of risk called the relative odds, also called the odds ratio (OR), is calculated instead. The OR ratio is interpreted in the same way as the relative risk.

		Ill	
		+	–
Exposed	+	A	B
	–	C	D

Illness Rate (Exposed) = A/(A + B) (%)
Illness Rate (Unexposed) = C/(C + D) (%)

Figure 3.2.3. Calculation of illness rates in a follow-up study.

If an investigator can show a positive exposure-response relationship (a greater likelihood of illness with more reported consumption of water), a greater assurance is provided that water was the cause of that illness. A positive exposure-response relationship tends to reinforce other significant findings of an investigation. An example of a positive exposure-response relationship is shown in Figure 3.2.5.

Laboratory Analyses

In addition to administering the questionnaire and gathering other data on potential exposures that may assist in determining whether illness was caused by water consumption, it is important for the investigator to attempt to determine the agent that causes illness. In half of the waterborne outbreaks reported between 1971- 1985, no causative agent was identified (2). Good laboratory support and timely investigation are necessary to identify etiologic agents.

At the Vermont Department of Health, fecal and sera samples are collected in most investigations of outbreaks that could be caused by a waterborne agent. Stool samples or rectal swabs are collected and

		Ill	
		+	−
Exposed	+	A	B
	−	C	D

$$\text{Relative Risk} = \frac{\dfrac{A}{(A+B)}}{\dfrac{C}{(C+D)}}$$

$$\text{Odds Ratio} = \frac{AD}{BC}$$

Figure 3.2.4. Calculations for measurement of risk.

transported in Cary Blair media. In addition, stool samples are collected in sterile containers that will be refrigerated at 5°C for possible virus identification. Finally, stool samples preserved in formalin are collected if parasites are considered a possible agent of illness. Acute and convalescent sera are drawn for antibody tests for the Norwalk-like viruses and other experimental antibody tests that are being developed for *Giardia*, *Campylobacter*, and other agents. At least 12 stool swabs, 12 stool samples, and 12 acute and convalescent sera samples are usually collected from both ill and well persons in an outbreak investigation. The Vermont Department of Health is capable of analyzing stool samples for bacterial and parasitic agents. Other diagnostic work is sent to laboratories outside of the state.

Investigation Caveats

There are many challenges to the investigation of waterborne outbreaks. Researchers have been justifiably concerned about exposure information collected from questionnaires. Specifically, the ability to accurately recall any event, such as water consumption, will decay over time. It is best to minimize this decay of information by surveying the population as soon after an outbreak as possible. It is recognized that responses provide, at best, estimates of water

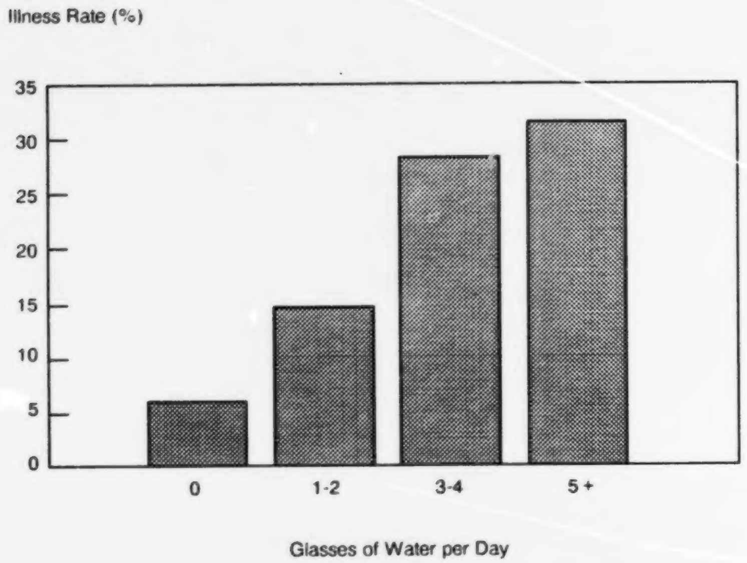


Figure 3.2.5. **Exposure-response relationships in an outbreak of waterborne illness.**

consumption and that answers are estimates and not precise measures of water exposure.

Diarrhea can be another confounding factor for an epidemiologic investigation. It is quite possible that investigators are not really testing the hypothesis that water consumption caused diarrhea as much as showing that ill people tend to drink more water than those who are well, to replace fluids. Questions concerning water consumption need to be specific about water exposure prior to the onset of the current illness.

Secondary person-to-person transmission may cloud an association between illness and the consumption of water. To analyze data in this situation, it may be advantageous to study only early outbreak cases if the agent is known to be spread easily from person to person. In addition, since many investigations really sample households through random-digit and door-to-door surveys, data should be analyzed by affected *households* instead of affected *persons*. This analysis will minimize the possible confounding effect of person-to-person transmission within households.

Although it may seem attractive to select persons in one township as a control for a township that has been affected by consuming contaminated water, there can be problems with this approach. The populations between the two towns should be similar except for exposure to contaminated water. If the comparison township has fewer children in day-care or has residents who are in a higher socioeconomic status, it may not be suitable as a comparison for the affected town. Any differences in illness rates between these two townships may be due to these possibly confounding factors instead of exposure to contaminated water. Similarly, if the comparison township has a greater number of children in day-care or has a lower socioeconomic status, one may have difficulty determining whether illness was caused by contaminated water in the affected township. The infectious agent could have been transmitted from person to person through affected day-cares instead of through contaminated water. Fortunately, there are now techniques to evaluate and control some forms of confounding after studies are conducted (3).

Conclusion

In an investigation of a potential waterborne outbreak, it is important to follow these steps:

- Obtain information about the nature and extent of illness
- Determine if water is a likely hypothesis
- Conduct an epidemiologic study to test the hypothesis
- Conduct an environmental study of the water
- Analyze the data
- Develop conclusions
- Institute control measures at appropriate points in the investigation

Thorough epidemiologic and environmental investigations are necessary for collecting scientific evidence that will show that contaminated water caused an outbreak of illness.

References

1. Rosenberg, M. L. A guide to the investigation of waterborne outbreaks. *J. AWWA.* 69: 4106-4110, 1977.

2. Centers for Disease Control. Water-related disease outbreaks, 1985. *Morbidity Mortality Weekly Report CDC Surveillance Summaries.* 37(55-2): 15-24, 1988.

3. Rothman, K. J. Stratified analysis. *In:* Modern Epidemiology. Little, Brown and Company, Boston, Massachusetts, 1986. p. 177.

Data Analysis: Estimating Risk

by:
Neal D. Traven
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh
Pittsburgh, Pennsylvania 15261
(412) 624-0097

Introduction

After investigators have amassed information about a suspected waterborne disease outbreak, these data must be analyzed statistically in order to test hypotheses about the data under study. Statistical analysis of questionnaire data collected from persons who may have been exposed to microorganisms suspected of causing disease is an essential component of the investigation of waterborne disease outbreaks.

Guidebooks for outbreak investigators often include such procedures as determination of the incubation period and graphing of the epidemic curve in their section on statistical analysis (1). Those techniques, however, are useful largely in identification of common source outbreaks and their possible causes rather than in evaluating statistical associations between the disease and environmental risk factors.

This chapter assumes that a disease-causing organism has been characterized and that the outbreak has been identified as arising from a common source. It also assumes that the collected data are accurate and unbiased, and that the questions asked of interviewees are appropriate and well-designed. Our focus is instead on the assessment of associations between the disease and other factors. The statistical tests discussed below can assist the investigator in estimating the strength of suspected associations with environmental exposures.

Among the topics covered in this report are point estimates of risk (rate difference, relative risk, and odds ratio) and the associated confidence intervals of these risk estimates. Equations for calculating these quantities are given, along with information which may aid the investigator in deciding which statistics to calculate in the analysis of a data set. Where appropriate, illustrative examples are presented from data taken from an actual waterborne disease outbreak investigation. In addition, the two types of statistical error will be defined. The interrelationship between sample size and statistical power, which is a function of Type II error, will be described and characterized.

Definitions and Notation

Before discussing epidemiologic data analytic methodologies, it is necessary to present some general notation that will be used throughout this chapter. Underlying much of epidemiologic data analysis is the fourfold table shown in Figure 3.3.1. In this generic form, both the disease and the environmental exposure under investigation are dichotomized. The letters *a*, *b*, *c*, and *d* represent the number of responses in each cell; the values n_1, n_2, m_1, and m_2 are the marginal totals for each category; and *N* is the total number of respondents in the entire analytic table.

		Disease		
		Yes	No	
Exposed	Yes	a	b	m_1
	No	c	d	m_2
		n_1	n_2	N

Figure 3.3.1. Epidemiologic data analysis: generic 2 × 2 table notation.

The essence of epidemiologic data analysis is *comparison*. To assess the significance of an epidemiologic association, one must establish a reference level with which to compare the exposure of interest. For example, nearly all heroin addicts drank milk in childhood, but it would be incorrect to conclude from this fact that drinking milk is associated with heroin addiction. One would probably find a similar milk consumption history among nonaddicts as well.

In making epidemiologic comparisons, it is essential to place the quantities being compared on an equal footing. Looking only at the raw numbers of diseased and healthy individuals, for example, can be misleading. Instead, epidemiologic comparisons are often drawn between *rates*, defined as the number of persons with a certain characteristic divided by the total population, in a specified time period (2). Such comparisons may also be drawn on *proportions*, in which the value in the numerator is included in the denominator (3). One can carry out both *difference* and *ratio* comparisons of rates; both forms offer important information about the magnitude of risk.

In these comparisons, the best single value estimate of the effect in question is called a *point estimate*. While the point estimate indicates the magnitude of the comparison, it does not take into account the degree of random variability in the observations. To allow for this variability, the researcher can estimate the effect using a range of values. This range is called the *confidence interval*, and the process of calculating it is *interval estimation* (4). The width of the confidence interval depends on both the variability of the data and an arbitrarily chosen level of confidence. A general formulation for any confidence interval is:

$$(\text{point estimate}) \pm (\text{z-score})\cdot(\text{std dev})$$

where *z-score* is the value of the Gaussian normal distribution corresponding to a preselected confidence level, and *std dev* is the standard deviation of the point estimate (the standard deviation, of course, is a measure of variability).

To apply the equations in this chapter, examples will be presented using data derived from an actual investigation of a waterborne disease outbreak. In this 1974 study (5), the causative agent was *Shigella sonnei*. A community whose well was contaminated with the organism was compared with a neighboring community served by an uncontaminated well. The fourfold table from which these examples are derived is displayed in Figure 3.3.2. The interval estimates in these numerical examples will all use 95 percent confidence intervals (z-score 1.96).

Epidemiologic Study Design

The hallmark of the epidemiologic study is analysis of the relationship between groups of persons with the disease condition under study and other persons, otherwise comparable to those persons, who are free of the disease. Figure 3.3.3 presents a typology of epidemiologic study designs proposed by Lilienfeld and Lilienfeld (6).

Experimental studies and trials are usually carried out to test the safety, efficacy, and utility of pharmaceutical products or other therapeutic modalities. Though experimental trials have been employed to determine infectious dose of some pathogenic organisms, this form of epidemiologic study will not be discussed in this chapter.

Most epidemiologic research studies are observational in design. The two major types of observational designs are *cohort* and *case-*

		Diarrheal Disease		
		Yes	No	
Affected Community	Yes	67	286	353
Control Community	No	16	276	292
		83	562	645

Figure 3.3.2. Epidemiologic data analysis: numerical example (*S. sonnei*)..

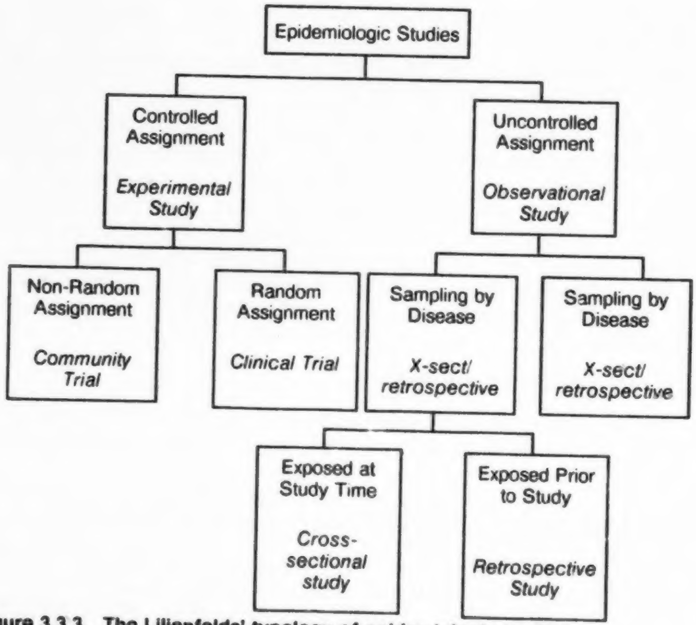

Epidemiologic Studies
Controlled Assignment
Experimental Study
Uncontrolled Assignment
Observational Study
Non-Random Assignment
Community Trial
Random Assignment
Clinical Trial
Sampling by Disease
X-sect/ retrospective
Sampling by Disease
X-sect/ retrospective
Exposed at Study Time
Cross-sectional study
Exposed Prior to Study
Retrospective Study

Figure 3.3.3. The Lilienfelds' typology of epidemiologic studies.
Adapted from (6).

control studies. These designs differ with respect to the theoretical populations from which subjects are drawn.

In a cohort study, participants are selected based on their risk factor status, then followed through time to determine their disease status at some future date; hence its alternative title, "prospective" study. At the beginning of a cohort study, all participants are free of observable disease.

In contrast, a case-control study samples diseased persons and comparable persons without the disease, then examines their personal histories to determine whether prior risk factor differences are associated with their current disease status. Case-control studies have also been called "retrospective" studies. Cross-sectional studies, in which the disease and the risk factor are examined at the same time, are analytically similar to the case-control design.

The Lilienfelds' typology is just one of several such frameworks put forward by epidemiologists. One might, for example, establish a cohort based on common exposure in the past, then follow this *historical cohort* into the present; this design is used regularly by occupational epidemiologists. The *nested case-control* design draws its cases and controls from the population base of a preexisting cohort study. In an *ecologic* study, the unit of measurement and analysis is aggregate data rather than information on specific individuals.

Statistical Tests

Chi-Square Test of Association

The standard statistical test used to analyze data in a fourfold table is the chi-square test of association. In this procedure, the sample chi-square is calculated and compared with the entries in a table of standard chi-square values. Using the notation introduced in Figure 3.3.1, chi-square can be computed by substituting into the following equation:

$$\chi^2 = \frac{(ad - bc)^2 \cdot N}{n_1 \cdot n_2 \cdot m_1 \cdot m_2} \qquad \text{(1 degree of freedom [d.f.])}$$

Algebraically equivalent formulas for computation of chi-square are available in any statistical textbook; suggested sources include Snedecor and Cochran (7) or Fleiss (8). All are variations on the concept of squaring the difference between the observed and "expected" values, and dividing by the expectation. It should be noted that the above chi-square equation differs from those found in most of these statistical texts in that it does not include the "correction for continuity." This quantity was introduced by Yates (9), and has been the subject of controversy in the statistical community for decades. In large studies, the Yates correction makes very little difference in the calculated chi-square statistic.

In the numerical example (Figure 3.3.2) the calculated chi-square is 25.98, indicating that the disease is strongly associated with the contaminated well (with the Yates correction the value is 24.79, very slightly lower than the uncorrected result).

Rate Differences

Outbreak investigators should be familiar with this statistical measure, because tables of "attack rates" are nothing more than sets of rate difference comparisons. The point estimate of the attack rate difference (RD_a) is calculated by simply subtracting the disease rate in the reference group from the disease rate in the affected population:

$$RD_a = (a/m_1) - (c/m_2)$$

Because it is a function of two proportions, the RD statistic ranges between -1 and +1; its null value is zero. Its distribution is symmetric about the null value.

For calculating the interval estimate of attack rate differences, the standard deviation term is derived as the sum of two binomial variances (3):

$$RD \pm 1.96 \cdot \sqrt{[(ab/m_1^3) + (cd/m_2^3)]}$$

It should be noted that there are methods other than the standard deviation approach for calculating interval estimates. Among them are Miettinen's test-based method (10) and the iterative method developed by Cornfield (11). The test-based method is easiest to calculate, but it has been shown to be unreliable in situations where the statistic is very distant from the null hypothesis value (12). Cornfield's method is more accurate, but its computational complexity requires the use of a computer and specialized software. The standard deviation method given in this chapter, then, is a good compromise choice between those of Miettinen and Cornfield.

In the numerical example, the calculated difference between the disease attack rates of the study communities is (0.190 - 0.055) = +0.135. The 95 percent confidence interval estimate covers the range (+0.086, +0.184). Because this interval does not contain zero, the null value under the hypothesis of no association, it is reasonable to conclude that there is a significant difference between the incidence rates in the two communities and that this difference is approximately 135 per 1,000 residents.

Relative Risk

While the RD statistic describes the difference between incidence in the exposed and control populations, the relative risk statistic (*RR*) consists of the ratio of those incidence rates. Its equation is:

$$RR = \frac{a/m_1}{c/m_2}$$

Relative risk takes values in the range from zero (when a is zero) to positive infinity (when c equals zero). The null hypothesis value is unity, at which point the incidence rates in the exposed and unexposed populations are equal.

In contrast to the rate difference, the distribution of the relative risk is asymmetrical. It is necessary to transform the statistic in some manner in order to calculate a standard deviation. After this step, one must then perform the inverse transformation to produce the confidence limits for the interval estimate of relative risk. These limits are symmetric about the point estimate in the transformed scale but are not symmetric after retransformation to the original scale.

The appropriate transformation in this situation is logarithmic. The natural log of the relative risk, or *ln(RR)*, is a linear function ranging from negative infinity to positive infinity with a mean of zero. Thus, Rothman's equation for calculating the interval estimate of the relative risk (3) is.

$$\exp\{\ln(RR) \pm 1.96 \cdot \sqrt{[(b/am_1) + (d/cm_2)]}\}$$

In the numerical example, the point estimate of the relative risk is 3.46; the incidence rate in the affected community is about three and one half times that of the unaffected community. The researcher calculates the standard deviation of ln(RR), computes the interval estimate of the transformed variable, and then takes the exponential of the confidence limits. This procedure results in a 95 percent confidence interval for relative risk with the range (2.05, 5.84). This interval does not include the null hypothesis value of unity, so one can conclude that there is a significantly increased risk of the disease in the study community with contaminated water.

Odds Ratio

In many situations, it is not appropriate to calculate the relative risk. The primary obstacle is that incidence rates cannot be calculated in cross-sectional or case-control studies, in which sampling is carried out based on disease status. In such studies, the sampling fractions for cases and controls are necessarily quite divergent; one always samples a far higher proportion of all diseased persons than of nondiseased persons (13).

The odds ratio (*OR*), defined below, is an extremely useful alternative to the relative risk. For rare diseases, it closely approximates RR. It can be determined from samples based on either disease status or exposure status, as long as it can be assumed that the study cases are representative of the diseased population, and that the controls represent the nondiseased general population (6).

$$OR = \frac{a \cdot d}{b \cdot c}$$

This statistic has gained favor in epidemiologic data analysis for a number of reasons. In addition to those outlined above, it is easy to compute, the variance of its logarithm is appealingly simple (see equation below), and the coefficients in logistic regression, an important multivariate statistical technique, are equivalent to odds ratios. The interval estimate of the OR is:

$$\exp\{\ln(OR) \pm 1.96 \cdot \sqrt{[(l/a) + (l/b) + (l/c) + (l/d)]}\}$$

The odds ratio for the numerical example equals 4.04. The 95 percent confidence interval, or interval estimate of the OR, covers the range (2.29, 7.15). Once again, this confidence interval does not contain the null hypothesis value, which is 1.00 for the odds ratio. Therefore, one concludes that the odds of diarrheal disease are significantly higher in the affected community than in the comparison community. The best estimate is that the probability of disease in the affected community is four times as high as in the unaffected community.

Sample Size and Statistical Power

An epidemiologic study should include enough subjects to control for two sources of statistical error. *Type I* or α-error occurs when one claims that an association exists between two factors (in this case, exposure and disease), when no such association truly exists. In *Type II* or β-error, the researcher claims that there is no association between the factors, when in actuality there is a real association. Of course, it is impossible to know the true state of association between the factors (if it were possible, there would be no reason to do the study!).

Standard statistical hypothesis testing is concerned with estimating the degree of α-error. In part, this concentration on Type I error is an artifact of the historical development of statistical theory. The turn-of-the-century founders of modern statistics (R.A. Fisher, Karl Pearson, and others) worked in agricultural research, where Type I error was far more important than Type II error; serious food shortages might result if the claim that a new fertilizer would improve crop yields turned out to be incorrect.

Statistical *power* is defined as the quantity, 1 - β. If a true association does exist in the population, then the power of the study is the probability of finding a *sample* odds ratio significantly different from the null value of unity.

Several equations are available for calculating the power of a sample (the details are beyond the scope of this chapter). The unknowns in such equations are α-error, β-error, minimum detectable odds ratio, and sample size. By fixing three of these quantities and solving for the fourth, the researcher could: 1) determine the required sample size for a given level of α-error, β-error, and minimum odds ratio; 2) calculate the power of the study to detect a given odds ratio at a selected level of α-error; or 3) estimate the smallest detectable odds ratio for a study with given α-error, β- error, and sample size.

Discussion

Choosing the right statistical tests for comparing and estimating disease risks is more an art than a science. In general, the odds ratio is preferable to the relative risk, since it is a valid statistic for both disease-based and exposure-based study designs. Its simplicity and its extendibility to multivariate analysis increase its utility in epidemiologic research.

In situations where it is appropriate to calculate disease incidence rates (i.e., when sampling with respect to the exposure variable), both the rate difference and either the relative risk or the odds ratio are appropriate statistical measures. In the numerical example in this chapter, sampling was undertaken based on the community of residence; the researchers were evaluating disease rates in areas with different water supply systems. Hence, there was justification for calculating both the RD and RR statistics in addition to the odds ratio (OR).

These methods for analyzing fourfold tables can be extended in many ways. There are epidemiologic methods for analyzing variables with more than two levels, for investigating the simultaneous effects of many variables, for studying interactions among variables, for matched analysis, and many more.

References

1. Bryan, F.L. Epidemiologic procedures for investigation of waterborne disease outbreaks. *In:* G.F. Craun (ed.), Waterborne Diseases in the United States. CRC Press, Boca Raton, Florida, 1986. p. 171.
2. Mausner, J.S. and Bahn, A.K. Epidemiology: An Introductory Text. W.B. Saunders Co., Philadelphia, Pennsylvania, 1974.
3. Last, J.M. A Dictionary of Epidemiology. 2nd edition. Oxford University Press, New York, New York, 1988.
4. Rothman, K.J. Modern Epidemiology. Little, Brown and Company, Boston, Massachusetts, 1986.
5. Weissman, J.B., Craun, G.F., Lawrence, D.N., Pollard, R.A., Saslaw, M.S., and Gangarosa, E.J. An epidemic of gastroenteritis traced to a contaminated public water supply. *Am. J. Epidemiol.* 103: 391, 1976.

6. Lilienfeld, A.M. and Lilienfeld, D.E. Foundations of Epidemiology. 2nd edition. Oxford University Press, New York, New York, 1980.

7. Snedecor, G.W. and Cochran, W.G. Statistical Methods. 7th edition. The Iowa State University Press, Ames, Iowa, 1980.

8. Fleiss, J.L. Statistical Methods for Rates and Proportions. 2nd edition. John Wiley & Sons, New York, New York, 1981.

9. Yates, F. Contingency tables involving small numbers and the chi-square test. *J Roy. Stat. Soc. Suppl.* 1: 217, 1934.

10. Miettinen, O.S. Estimability and estimation in case-referent studies. *Am. J. Epidemiol.* 103: 226, 1976.

11. Cornfield, J. A statistical problem arising from retrospective studies. *In*: Proceedings of the Third Berkeley Symposium, Volume IV. University of California Press, Berkeley, California, 1956. p. 135.

12. Gart, J.J. and Thomas, D.G. The performance of three approximate confidence limit methods for the odds ratio. *Am. J. Epidemiol.* 115: 453, 1982.

13. Schlesselman, J.J. Case-Control Studies: Design, Conduct, Analysis. Oxford University Press, New York, New York, 1982.

Avoiding Bias: Systematic and Random Error

by: Patricia A. Murphy
Health Effects Research Laboratory
Human Studies Division
U.S. Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, OH 45268
(513) 569-7226

Introduction

The investigation and control of communicable disease epidemics has long been considered the paradigm of epidemiologic study. Recent methodologic developments and a greater emphasis on the study of chronic, noninfectious diseases have helped to force a change in epidemiology. Along with this shift there has been a trend to analyze data from an epidemic investigation using some of the more sophisticated statistical procedures developed for the study of chronic disease. Data that may be appropriate for confirming the existence of an outbreak, however, are not always adequate for further robust statistical analysis. As this trend in analysis becomes increasingly common, it is imperative to be aware of the potential limitations of any inferences made in epidemiologic investigation where findings are sometimes based on a small, biased sample of the population.

It is well recognized that investigations of waterborne disease transmission almost always take place in the public eye. The epidemiologist must make rapid decisions regarding control of an epidemic, and the decisions may later affect inferences that can be drawn from the available data. In particular, the expedient collection of information may result in certain biases or error. Although all outbreak situations have unique characteristics, certain information must always be collected. Investigators must begin to develop a standardized way to collect this information (for example, amount of water consumed) and minimize measurement error in epidemiology studies wherever possible. Etiologic research standards must be applied to all data, including those from outbreak investigations, that are analyzed and presented as if they were originally collected for that purpose.

All epidemiologic studies, whether basic etiologic research, compilations of descriptive statistics, or outbreak investigations, require some type of quantitative measurement. For example, the purpose may be to define the prevalence of a gastrointestinal illness in residents of a nursing home or to determine the amount of contaminated drinking water consumed by persons with and without a particular illness. A major goal of most epidemiologic investigations is to make a quantitative statement about the relationship between a particular exposure and a particular disease in a population. In order to draw accurate and valid conclusions from this work, investigators should strive to make these measurements as error-free as realistically possible in any given situation. This goal will be realized only through awareness of the major sources of error that exist, the likelihood of their occurrence, and the manner in which measurements may be affected. This article presents an overview of some of the major sources of measurement error that can bias epidemiologic study results and interpretation.

Background and Definitions

In an epidemiologic investigation, the process of quantifying the relationship between an exposure and a disease is referred to as effect estimation. The usual effect estimates that are calculated include the rate ratio or relative risk (RR), the rate difference (RD), and the odds ratio (OR). Methods for their calculation are detailed in the preceding article by Traven (1). If the measured effect differs in some way from its true value it is said to be biased. The primary considerations for evaluating the accuracy of an epidemiologic measure are precision, or lack of random error, and validity, or lack of systematic error. Within this realm, there are many factors that may potentially, and in some cases assuredly, lead investigators to make a biased estimate. A brief outline of some of the recent developments in epidemiology over the last two decades will help place in perspective the recognition of the great role that bias may play in effect estimation.

Historically, most epidemiologic research involved investigations of epidemics of infectious disease. Great improvements in social and medical well-being during this century forced a shift away from the study of infectious diseases and toward the development of the ever-growing field of chronic diseases such as cancer and cardiovascular disease. This shift has been instrumental in the development of newer and more sophisticated techniques for the analysis of data generated during an investigation. It has also contributed to a greater understanding of the conceptual basis for specific study designs, particularly the case-control study. Case-control studies were employed with growing frequency, and as the popularity of this approach to research grew, it was realized that certain features inherent in a retrospective approach to the study of the causes of disease created a great potential for the acquisition of biased information. This realization led many scientists to believe that there was something inherently wrong or bad about retrospectively based investigations. In fact, what should have been questioned was not the retrospective approach in general, but rather the unfortunate lack of attention to details of study design by persons inexperienced with the use of this type of study. An enormous controversy grew among leading epidemiologists, and grave reservations were expressed as to the validity of this design option.

In an effort to openly discuss the problems and reservations about case-control studies specifically and retrospective study designs in general, a symposium was held in 1978, and a special issue of the *Journal of Chronic Diseases* (2) was devoted to the proceedings and discussions. One major point of contention related to the many places in a study where some form of bias could be operating. There was also grave concern about the scientific quality of many of the studies appearing in the literature and the apparent lack of standards for high quality case-control research. It was at this symposium that Sackett (3) presented a paper on bias in analytic research, wherein he outlined a catalogue of 35 biases that may crop up in a retrospective study. This was the first comprehensive attempt at such an endeavor and marked what was probably a turning point in the relatively new field of chronic disease epidemiology. Although many investigators were aware of the existence of and potential for bias to occur, it was apparent that most researchers had not thought of the impact of bias as being quite so far-reaching. It was at the same symposium that Ibrahim and Spitzer (4) succinctly classified bias into three major categories of systematic error: selection bias, information bias, and confounding bias. This classification has received widespread acceptance and most modern textbooks of epidemiologic methods present the concept in this way (5-8). The latest edition of the Dictionary of Epidemiology (9) lists 26 definitions of specific biases and, like Sackett's list (3), they can all be classified into one of these three groups. Empiric investigations of the impact of bias in epidemiologic research have further demonstrated the need for consideration of all sources of error in investigations (10,11). The results from waterborne outbreak investigations can also be affected by the same kinds of error, some of which will be considered in the next section.

Precision and Validity

A thorough epidemiologic analysis must assess bias, confounding, causation, and chance as explanations for the observed results (12). Implicit is the assessment of the various components of error present in effect estimators and occurrence measures. This section briefly reviews the concepts of systematic and random error and their likely impact on epidemiologic measures derived during the course of an outbreak investigation. Whenever possible, examples are specifically geared to address the potential for occurrence in a waterborne disease outbreak investigation.

Precision

Precision refers to the lack of random error in a measurement. Random error has many components, but the major contribution comes from the process of selecting subjects for inclusion in a study. This is known as sampling error (6). Even if all cases of disease are included as part of an investigation, the subjects are always considered to be a sample and there will be some degree of sampling error present (6). One measure of precision can be obtained by calculating a confidence interval (CI) of an arbitrarily chosen significance, usually constructed as a 95 percent CI. A wide CI indicates a lack of precision in the estimate. CIs are occasionally interpreted as tests of statistical significance but their primary function, assessing measurement variability, should not be forgotten. The p-value is usually reported in an outbreak investigation as a measure of statistical significance. Although often cited as evidence that a particular hypothesis has been proved, the p-value is not the probability that the tested hypothesis is true, but rather the probability that chance alone would produce a difference between the compared groups at least as big as the one observed (5). A small p-value indicates that it is unlikely that chance or random error is the sole explanation for a study's findings, but it reveals nothing about the role of other forms of error.

The most common way to increase the precision of a study is to increase the sample size. Usually, this is not possible in an outbreak investigation, as there will be only a finite number of cases that come to medical attention and are included as study subjects. This limitation cannot be changed; however, an investigator can make more efficient use of the available information by either matching, increasing the ratio of controls to cases, or appropriately restricting the study population. These design options will help to increase the precision of measurement when accompanied by appropriate analytic treatment. There will always be some degree of imprecision associated with any epidemiologic measure. An investigator should therefore provide a quantitative estimate of this imprecision and temper all interpretations accordingly.

Validity

When a study is considered "valid," it usually means that there is a lack of systematic error affecting the results. It should be thought of in terms of both internal validity and external validity, with the latter referring to the extent to which the study results can be generalized to another population. Before the results are projected in this way, the internal validity must be assessed. As mentioned in the introduction, numerous biases can occur and detract from the internal validity of a study; these are generally classified as selection bias, information bias, and confounding bias.

A *selection bias* results from procedures used to select subjects that lead to an effect estimate among the included subjects differing from that occurring in the entire population of available cases (6). There are many ways in which selection bias can occur in an epidemiologic study. The common element underlying all selection bias is that the relation between exposure and disease is somehow different for those who participate and those who would be theoretically eligible for study but do not participate. Some degree of selection bias is likely to occur in an epidemic investigation. For example, many if not all cases have volunteered to be included for study, simply by going to their physician or the emergency room for treatment of their illness. Less severely ill cases may not seek any medical care and would never be recognized as cases. This could lead to both an underestimate of the total population affected during an outbreak and, perhaps to a distortion of the proportions possessing various risk factors of interest. The investigator should anticipate the various selection biases that could be operating in any particular study and provide an objective assessment both of the likelihood of occurrence and the way in which results could be affected.

The second major category of bias likely to be present in any epidemiologic study is *information bias*. The inherent imprecision of the retrospective questionnaire as an instrument for data collection can be a major cause of this form of bias. If the information obtained from the study participants is influenced by the group to which the participant belongs (e.g., a case or a control, exposed or not exposed), validity will be compromised (5). Information bias can occur whenever there are errors in the classification of study subjects, but the consequences of the bias will be very different depending on how it occurs. For the sake of simplicity, assume that disease classification is perfect, i.e., all persons classified as cases for the purposes of study are truly cases and those classified as controls truly do not have disease, and that errors occur only when assessing exposure. This is another way of stating that the sensitivity and specificity of the disease measure is 100 percent. Any time the sensitivity and specificity of a measure is less than 100 percent, there will be some misclassification. If the exposure misclassification is independent of the person's disease classification, then the misclassification is said to be nondifferential or random. On the other hand, if one group is more likely to be misclassified than the other, the misclassification is said to be differential or systematic.

It is well known that random misclassification always results in the OR being biased toward the null, resulting in an underestimate of the association (10). When the misclassification turns out to be systematic, however, it is not easy to predict either the magnitude or direction of bias in the OR. There are formulas available for correcting biased ORs, but it is necessary to know the sensitivity and specificity of the measure. This information generally is not available. Given the paucity of data for correcting estimates, the superior approach is clearly to try to anticipate and minimize misclassification.

Perhaps the most well-known type of information bias is referred to as *recall bias*. Recall bias is a distinct possibility in any study where information on past exposures is obtained. If cases are likely to recall the same event differently than the controls, recall bias will result. It is not hard to imagine examples of bias of this type. For example, by the time a case-control study is conducted during an outbreak investigation, it is likely that water has already been implicated as the vehicle of transmission (13). Since cases know their disease is waterborne, they may erroneously recall consuming larger quantities of water than they actually did. This type of recall bias could result in the appearance of a dramatic dose-response related to number of glasses of water consumed. On the other hand, it could also mask a real dose-response that truly does exist. It is up to the individual investigator to assess the likelihood of any bias occurring and the most likely way in which the results may be distorted. It is likely that any attempt to measure retrospectively the amount of fluid consumed during a particular point in time will be biased to some degree. Investigators need to recognize the limitations of these kinds of data to avoid overinterpreting findings. The situation may become further complicated when biased exposure data are used for sophisticated statistical modeling such as multiple logistic regression. The application of these techniques may possibly result in a further distortion of the effect estimate with unwarranted credence subsequently given to the findings. These are but a few of the ways that misclassification can affect study results.

The third major category of bias to be considered is that of *confounding bias*. The concept of confounding is an extremely important one in modern epidemiology and requires more attention than space permits in this chapter. The interested investigator is referred to one of the recent textbooks in epidemiology for a complete discussion of the issue (5-8). Rothman (6) has defined confounding as a mixing of effects: the estimate of the effect of the exposure is distorted because it is mixed with the effect of an extraneous factor. The distortion can be large or small, and it can lead to both over- and underestimation of an effect depending on the direction of the associations the confounder has with exposure and disease. It is possible for confounding even to change the apparent direction of an effect (6). Investigators should always anticipate confounding and collect appropriate data to control for its effects during the analysis if necessary. Matching is one way to control for confounding at the design stage but will not be appropriate or feasible in every case. In order for the matching to be effective, investigators must also use appropriate analysis techniques. Stratification of the data is the most straightforward way of controlling and assessing confounding (1) in the analysis stage, although other techniques such as multivariate modeling are frequently used. The appropriateness of any one means of controlling confounding will always be study-dependent and must be assessed on an individual basis.

Probably the most important thing to remember is that the presence of bias in and of itself does not necessarily invalidate an epidemiologic study. It should be incumbent on any investigator to thoroughly examine the methods employed in the study and identify areas where certain biases may exist. The investigator should anticipate what biases might occur, try to prevent or control them, and always try to estimate at least the direction of bias that might be present. Selection and information biases always must be anticipated and prevented since they cannot be controlled statistically during the analysis. Confounding can be totally prevented in many instances, but it can usually be controlled analytically if appropriate data have been collected.

Discussion

Epidemiologic research in the midst of an outbreak situation is at best a formidable task. Investigators of these epidemics should be commended for the quality of the data collected in the heat of intense public scrutiny and under extreme constraints of time. The fact that an outbreak will only leave a certain number of cases for epidemiologic investigation means that one must accept the fact that any measures of disease occurrence or association (RD, RR, OR) will be somewhat imprecise due to the limited sample size. The lack of precision, however, should not influence unduly the credibility of the results. The investigator should always be aware of the inherent limitations of small sample sizes and, wherever feasible, try to make more efficient use of the limited data points available. Some estimate of the degree of imprecision of an OR or RR, such as a CI, should always be calculated.

A trend toward more statistically sophisticated analysis of outbreak data has been noted and will most likely continue. The anticipation of this type of analysis should lead to more attention being paid to collecting all the data needed for a thorough assessment of bias and confounding necessary to reach a valid conclusion. Only by recognizing the potential for occurrence can flaws be either prevented or detected and corrected so that study inferences will be on target. Every epidemiologic study, regardless of purpose, should be held to certain accepted standards if epidemiologists are to continue to maintain their well-deserved place as the basic scientists of public health.

References

1. Traven, N.D. Data analysis: estimating risk. In: G.F. Craun (ed.), Surveillance and Investigation of Waterborne Disease Outbreaks. U.S. EPA, Cincinnati, OH. 1970.
2. Spitzer, W.O. (ed.). The case-control study: consensus and controversy. *J. Chron. Dis.* 32:1-144, 1979.
3. Sackett, D.L. Bias in analytic research. *J. Chron. Dis.* 32:51-63, 1979.
4. Ibrahim, M.A. and Spitzer, W.O. The case-control study: the problem and the prospect. *J. Chron Dis.* 32:139-144, 1979.
5. Ahlbom, A. and Norell, S. Introduction to Modern Epidemiology. Epidemiology Resources, Inc., Chestnut Hill, MA, 1984.
6. Rothman, K.J. Modern Epidemiology. Little, Brown and Company, Boston, MA, 1986.
7. Kelsey, J.L., Thompson, W.D., and Evans, A.S. Methods in Observational Epidemiology. Oxford University Press, New York, NY, 1986.
8. Kleinbaum, D.G., Kupper, L.L., and Morgenstern, H. Epidemiologic Research--Principles and Quantitative Methods, Lifetime Learning Publications, Belmont, CA, 1982.
9. Last, J.M. (ed). A Dictionary of Epidemiology, 2nd edition, Oxford University Press, New York, NY, 1988, pp. 13-16.
10. Copeland, K.T., Checkoway, H., McMichael, A.J., and Holbrook, R.H. Bias due to misclassification in the estimation of relative risk. *Am. J. Epidemiol.* 105:488-495, 1977.
11. Greenland, S. The effect of misclassification in the presence of covariates. *Am. J. Epidemiol.* 112:564-69, 1980.
12. Cole, P. The evolving case-control study. *J. Chron. Dis.* 32:15-27, 1979.
13. Bryan, F.L. Epidemiologic procedures for investigation of waterborne disease outbreaks. Chapter 7. *In:* G.F. Craun (ed.), Waterborne Diseases in the United States, CRC Press, Boca Raton, Florida, 1986, pp. 171-193.

Predicting Exposure to Water Contaminants in Distribution Systems

by: Robert M. Clark
Drinking Water Research Division
Risk Reduction Engineering Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268
(513) 569-7201

Introduction

Although the Safe Drinking Water Act (SDWA) and its amendments clearly specify that MCLs shall be met at the consumer's tap, most regulatory concern has been focused on water as it leaves the treatment plant before entering the distribution system. The only SDWA regulations that emphasize system sampling are those that deal with microbiological contamination and total trihalomethanes.

There is, however, growing interest in determining the factors that cause water quality variations in drinking water distribution systems. Finished water may change in quality before it reaches the user, due to chemical or biological transformations or due to a loss of system integrity. Water distribution systems frequently draw water from multiple sources, such as a combination of wells, or different surface sources, or both. Mixing of water from different sources takes place within the distribution system, and is a function of complex system hydraulics. For all of these reasons the quality of water delivered to the consumer may vary spatially and temporally within the distribution system (1).

To understand water quality variations in distribution systems requires a knowledge of several factors, including the hydraulic behavior and the contaminant behavior of the system. It is almost impossible to study these factors directly because the scope and complexity of distribution systems are so great. Frequently, hydraulic models are used to analyze water system operation. Models can also be used to predict spatial distributions of waterborne contaminants in distribution systems. Flow patterns in distribution systems can be highly variable and these patterns can have significant impact on the way contaminants are dispersed in a network. This paper will discuss the development of a set of models for predicting propagation of contaminants in distribution systems including a steady-state model, a contaminant-dispersion model, a travel-time diversity index, and a dynamic water quality model for contaminant propagation. These models will hopefully lend insight into factors affecting variations in water quality in distribution systems. The models are developed and verified in the context of a case study conducted by U.S. EPA with the North Penn Water Authority (NPWA).

North Penn Water Authority

Until recently little attention has been paid to the problem of water quality in distribution systems. However, as concern over distribution system water quality has increased, more attention has been paid to the factors that effect water quality variations (2).

To provide the basis for studying changes in water quality as a result of mixing in distribution systems, a cooperative agreement was initiated between the North Penn Water Authority, Landsdale, Pennsylvania, and the U.S. EPA's Drinking Water Research Division.

The NPWA serves 14,500 customers in 10 municipalities with an average of 5 million gallons (MGD) of water per day. Water sources include a 1 MGD treated surface water source purchased from the Keystone Water Company and 4 MGD from the 40 wells operated by NPWA. Figure 3.5.1 is a schematic representation of the 225 miles of pipe in the NPWA distribution system, showing the location of wells, the Keystone "tie-in" and the three pressure zones: Souderton zone, Lansdale low zone, and Hillcrest zone.

Surface water enters the NPWA system at the Keystone tie-in. The rate of flow into the system is determined by the elevation of the tank in the Keystone system, and by a throttling valve at the tie-in. Flow is monitored continuously and is relatively constant. Water flows into the Lansdale low- pressure zone and from there enters the Lawn Avenue tank, from which it is pumped into the Souderton zone. Additional water, from the Hillcrest pressure zone, enters the Lansdale system at the Office Hillcrest transfer point (Office Stand Pipe); this water is solely derived from wells in the Hillcrest zone. Except for unusual and extreme circumstances, such as fire or main breaks, water does not flow from the Souderton zone into the Lansdale low zone, nor from Lansdale low into Hillcrest.

There are distinct chemical characteristics of Keystone water compared with well waters. Keystone water contains total trihalomethanes (TTHM) at significantly higher levels than well water. Certain wells show the occurrence of trichloroethylene (TCE) and/or cis-1,2-dichloroethylene (cis-1,2-DCE). Inorganic chemicals also vary from well to well, and between the wells and Keystone. Trihalomethanes are a result of the interaction of precursor material in the raw water with chlorine. After they reach a given level they act

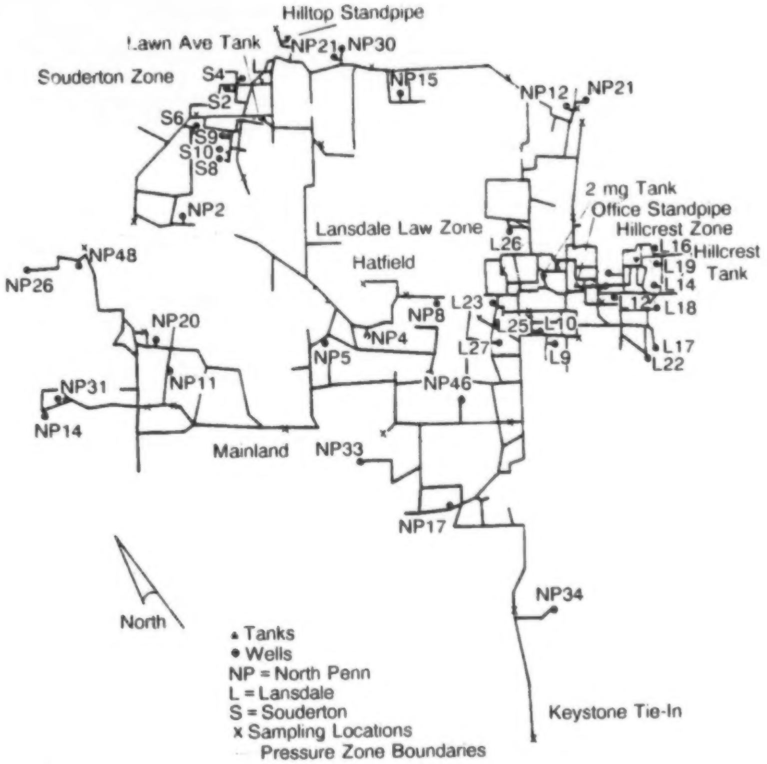

Hilltop Standpipe
Lawn Ave Tank
NP21
NP30
Souderton Zone
S4
S2
S6
S9
S10
S8
NP15
NP12
NP21
2 mg Tank
Office Standpipe
Hillcrest Zone
Hillcrest Tank
NP2
Lansdale Law Zone
L26
L16
L19
L14
L18
L12
NP48
NP26
Hatfield
NP8
L23
NP20
NP4
L25
L10
L27
L9
L17
L22
NP5
NP31
NP11
NP46
NP14
Mainland
NP33
NP17
North
NP34
Keystone Tie-In
▲ Tanks
● Wells
NP = North Penn
L = Lansdale
S = Souderton
x Sampling Locations
Pressure Zone Boundaries

Figure 3.5.1. North Penn Water Authority distribution system.

as conservative substances. This is the case for TTHMs at the Keystone tie-in.

The NPWA distribution system was modeled in a network representation consisting of 528 links and 456 nodes. Water demands for modeling represented conditions during May-June 1984. The network hydraulic model used was the WADISO Model, which contains provisions for both steady-state and quasi-dynamic hydraulic modeling (extended period simulation) (3). In the first phase of the study, steady-state modeling was used, and included limited field calibration and validation of the model. The model was employed to study the overall sensitivity of the system to well pumpage, demand, and other factors. This study resulted in the development of a number of typical flow "scenarios." Researchers developed a "base" scenario, representing a typical situation with "wells on" and "tanks filling," with average demand as well as alternative scenarios which represented other typical demand and well status scenarios.

The modeling effort revealed portions of the system which were subject to changes in flow directions under different demand conditions. Figure 3.5.2 depicts the spatial distribution of the percentage of water in the Landsdale low zone from the Keystone supply under the base scenario.

Steady-State Prediction of Contaminant Distribution

Engineering analysis of water distribution systems is frequently limited to the solution of the hydraulic network problem, i.e., given the physical characteristics of a distribution system and the demands at nodes, the flows in links (the sections of pipe between nodes) and the head at all nodes of the network are determined. The problem is formulated as a set of simultaneous nonlinear equations, for which there are a number of well-known solution methods (1,5).

In addition to the hydraulic analysis problem, however, there are other problems of interest in water distribution systems. First, the calculation of mixing water from different sources in the system is of concern, particularly where sources of different water quality may exist. Secondly, calculation of travel time from a source to any demand node in the network may be of use in investigating waterborne disease outbreaks (determining which populations were exposed to a higher concentration of a contaminant), or examining time dependent quality parameters, such as trihalomethane levels. A third area of increasing interest is that of economic analysis and "cost of service" pricing, in which the allocated cost of water to various points within the system is calculated, and priced accordingly (similar in concept to the pricing of long-distance telephone calls, or airline tickets, based on distance and volume of use).

For each of these three types of problems, a solution variable can be defined. For the mixing problem, the variable of interest is the concentration of a particular constituent within the water. For the travel time problem, the variable is the "age" of the water, assuming that the water is of some defined age at each source in the system. For the cost problem, the variable is unit cost (e.g., $/cubic foot/sec).

The problems are formulated by assuming that a steady-state hydraulic solution of the system is known, i.e., all flows throughout the system are known and satisfy a mass balance at the nodes. Then, as each element of water proceeds from a source to a demand node, it may undergo a transformation as it passes along each link and mixes with water from other portions of the network at the nodes. By assuming complete mixing at each node, a linear equation in the concentration variable and the known flows can be written for each node, based on conservation of mass principles. With the unknown boundary

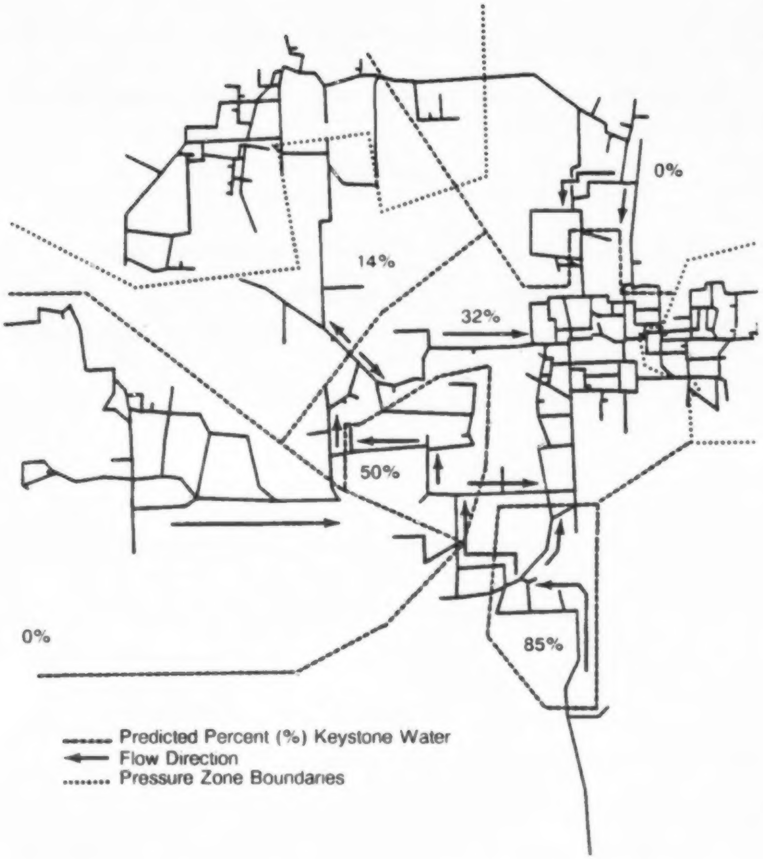

0%
14%
32%
50%
0%
85%
Predicted Percent (%) Keystone Water
Flow Direction
Pressure Zone Boundaries

Figure 3.5.2. Percent Keystone Water – average conditions scenario.

conditions at source to the network, equations in each of the unknown nodal variables can be written and solved by standard methods.

By appropriate definition of the variables, a general formulation for each of the three generic types of problems (concentration, travel time, and cost) can be developed. Each of the three types of problems can be represented by similar equation forms. For the concentration problem, there is neither a link- nor node-associated additive value.

For the travel time problem, there is a link-associated added value (the link travel time), and for the cost problem, there are both link- and node-associated fixed cost values. Males *et al.* present more complete analysis of this approach (5).

The general formulation at any node i, for all three types of problem, can be expressed as:

$$P^i = \frac{[\sum(\{Q_{ij} \cdot \ P_i\} + dlLV_{ij}\}) + \{d2 \cdot \ NV_i\} + \{S_i \cdot \ SP_i\}]}{I^i} \qquad (1)$$

where the sum is taken for all j such that flow is from j to i, and:

P^i = appropriate node-related parameter

LV_{ij} = corresponding line-associated value for link ij (cost and travel time solution)

NV^i = corresponding node-associated value for node i (cost solution)

S^i = external source flow into node i

SP^i = external boundary condition parameter value at node i

dl = 0 for concentration problem and 1 for cost and travel time problems

d2 = 0 for concentration and travel time problem and l for cost problem

I^i = total inflow to node i

Q_{ij} = flow from node j to node i

This solution technique is called Solver (5).

Numeric Example

A simplified network can be used to demonstrate how the equations are set up. Assume a two-source network consisting of five links and four nodes, as shown in Figure 3.5.3. It is assumed that the flows have previously been determined by hydraulic analysis, such that continuity is maintained at all nodes. Figure 3.5.3 shows the link-associated flows, flows from the two external sources, and the node demands. The concentration case is illustrated below, assuming that the concentration at source 1 is SC_1, and the concentration at source 2 is SC_2. Observing the flows at each node, and the associated flow directions, the following equations can be written for each of the four nodes:

$$C1 = \frac{(F_1 \bullet SC_1)}{F_1} \qquad (2)$$

$$C2 = \frac{(F_3 \bullet C_1 + F_8 \bullet C_3)}{F_3 + F_8} \qquad (3)$$

$$C3 = \frac{(F_{11} \bullet SC_2)}{F_{11}} \qquad (4)$$

$$C4 = \frac{(F_4 \bullet C_1 + F_7 \bullet C_2 + F_9 \bullet C_3)}{F_3 + F_7 + F_9} \qquad (5)$$

Thus, there are four equations in four unknowns that can be solved given the known values of the boundary conditions, SC_1 and SC_2. In this simplified case, these equations can be solved by direct substitution, whereas in more complex cases, matrix methods are applied. As mentioned earlier, once the network has been defined, the WADISO hydraulic model can be applied under "base" conditions to determine flows to the network links. The "Solver" component of EPA's Water Supply Simulation Model (WSSM) was applied to the steady-state hydraulic solution using the known concentrations in the Keystone water sources (6). An attempt was made to "match" the distribution of historical total TTHM data. Historical TTHM data from April 1984 and April 1985 were used to develop average concentrations for TTHMs as shown in Figure 3.5.4. Averages are based on 126 sampling results from 29 sites with 5 results available at most sites, and 52 results available at the Keystone tie-in. A TTHM level at the Keystone supply model was set at a value equal to that found in historical data. No bromoform was found in the TTHMs from Keystone but was found in the distribution system samples at approximately 10 g/L. Bromoform levels were most likely due to bromide in the well water. Therefore TTHMs at the 10 g/L level were assumed as coming from the wells.

Table 3.5.1 contains representative water quality values at Keystone in the distribution system and at selected wells. In the "base" scenario, it was assumed that water was flowing into the elevated Chestnut Street and Lawn Avenue elevated tanks. Tanks obviously have an impact on water quality in a distribution system because they may tend to diffuse contaminant concentrations. This effect is mitigated by assumption of a steady-state model but is extremely important in a dynamic model. Predicted values in Figure 3.5.4 show good agreement with the actual surveillance data.

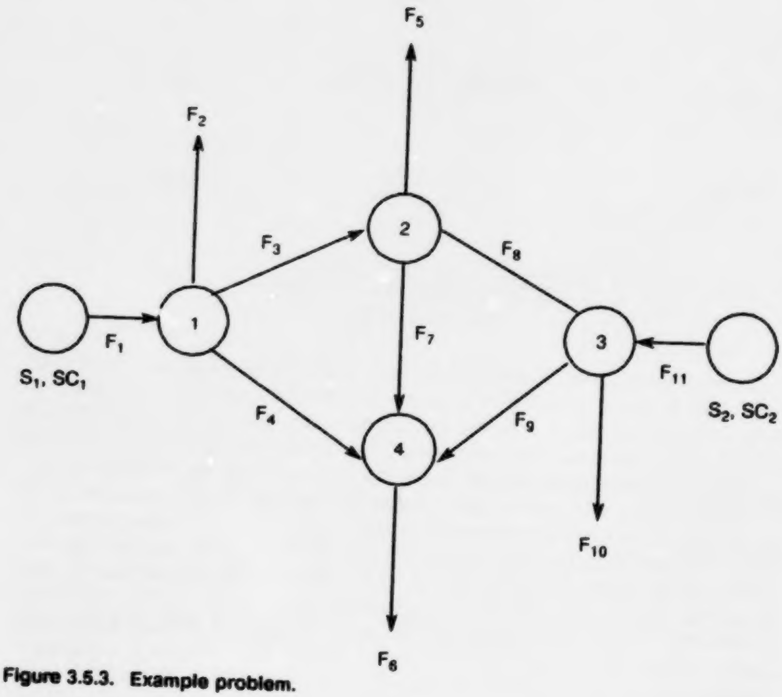


Figure 3.5.3. Example problem.

Dynamic Variations in Water Quality Data

To investigate the nature of water quality variability under dynamic conditions within the system, a sampling program was conducted at six sites. Sampling sites were selected based on spatial variations determined from this historical data and modeling results. Figure 3.5.5 shows the various sampling points used in the field study, and Figure 3.5.6 depicts the results of the intensive sampling program using TTHMs as a tracer. There is significant variation within the 36-hour period for given points, and there is significant variation within the system itself. Figure 3.5.6 also depicts the variation in hardness at these same points (hardness is primarily associated with flow from the wells). At the Mainland sampling point, a flushing back and forth of water between the surface source and the well sources can be seen. The peaks of the TTHMs at Mainland are approximately 12 hours out of phase with the peaks from the wells, indicating that water flow at this point is affected by the surface and ground-water sources.

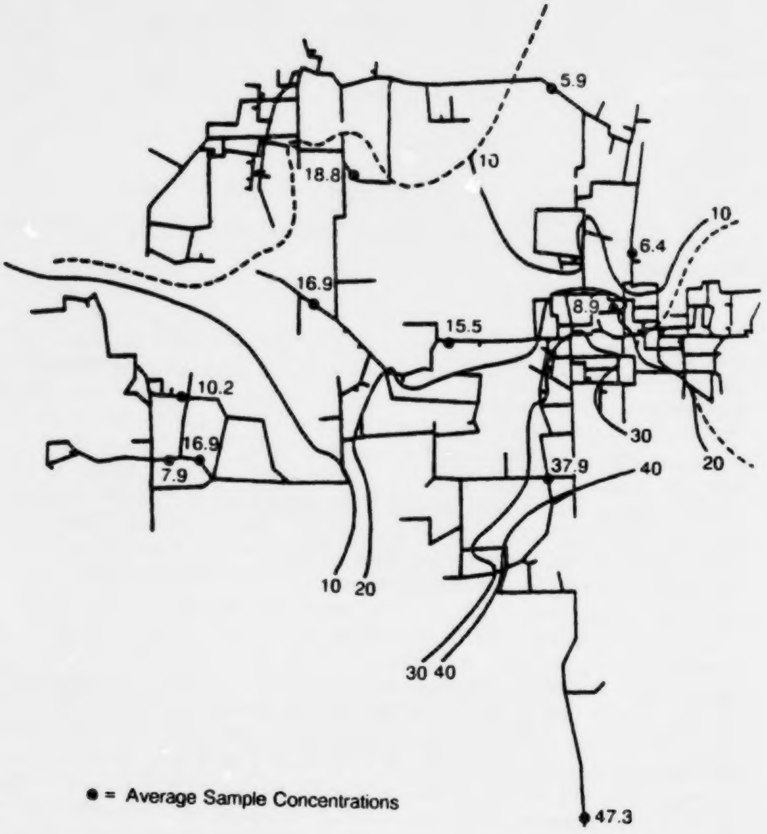

5.9
10
18.8
10
6.4
16.9
8.9
15.5
10.2
30
16.9
7.9
37.9
40
20
10
20
30
40
● = Average Sample Concentrations
47.3

Figure 3.5.4. Predicted versus actual trihalomethane concentrations . (Note: Contours based on average demand with wells on [modeled conditions]).

These results point out the problems in attempting to predict a dynamic situation using a steady-state approach. The average TTHM data represent long-term averages taken over a number of years, at different times of the day. The pattern of flow in the NPWA distribution system varies during the course of a day, as wells cycle off and on. Late at night, when most wells are off, Keystone water is the primary source of supply for the Lansdale low-pressure zone system,

Table 3.5.1. North Penn Water Quality Based on 1984 Data

Quality Factor (l)	Minimum (2)	Maximum (3)	Minimum (4)	Maximum (5)	Minimum (6)	Maximum (7)
Chloride (mg/L)	9	152	14	93	30	30
Hardness (mg/L)	100	440	156	297	124	190
Sulfate (mg/L)	9	242	23	125	36	73
TTHM (µg/L)	[a]	[a]	0.50	37.9	10.3	131.0

[a] Not measured.

and this water passes into the eastern and northern portions of the system, where it can enter the Lawn Avenue tank. During times of the day when Lawn Avenue tank water enters the Lansdale low system, this water can be expected to contribute TTHM (due to the origin of at least a portion of this water from Keystone when Lawn Avenue is filling). The pilot sampling run of November 14-15, 1985, shows a range during the sample period from a maximum TTHM value of 36 g/L to a minimum of 13 g/L, at the inlet to Lawn Avenue. This range is consistent with the historical data of Figure 3.5.4, where a value of 18.8 g/L is shown in the neighborhood of Lawn Avenue. Data from the pilot sampling run, taken at the Mainland sampling site, shown on Figure 3.5.6, shows TTHM values in the range from 9 to 34 g/L, with significant variation. As concluded by the analysis of the pilot sampling run, Mainland receives water from the wells in Harleysville (NP14, NP31, NP11, NP20, NP26, NP48) during the times of day when they are pumping, and from Keystone in the early morning hours. Historical data shows levels of TTHM of 2 to 6 g/L in this area, as compared to the model prediction (with wells on) of 0 g/L. The model does show, however, that the zone of blending of Keystone and well water is fairly close to Mainland; thus, the variations predicted by the model are close to the actual ones. The Mainland site was selected primarily to test this hypothesis in the pilot sampling run.

While the initial steady-state modeling did provide useful information, so far as design of the pilot sampling study was concerned, it was obvious that the ability to model the dynamic nature of the complex NPWA system with a single steady-state predictive model is limited. Accordingly, an attempt was made to extend the steady-state predictive principles to "a sequential steady-state" modeling approach for the situations encountered in the pilot sampling run. The following section describes the development of this approach.

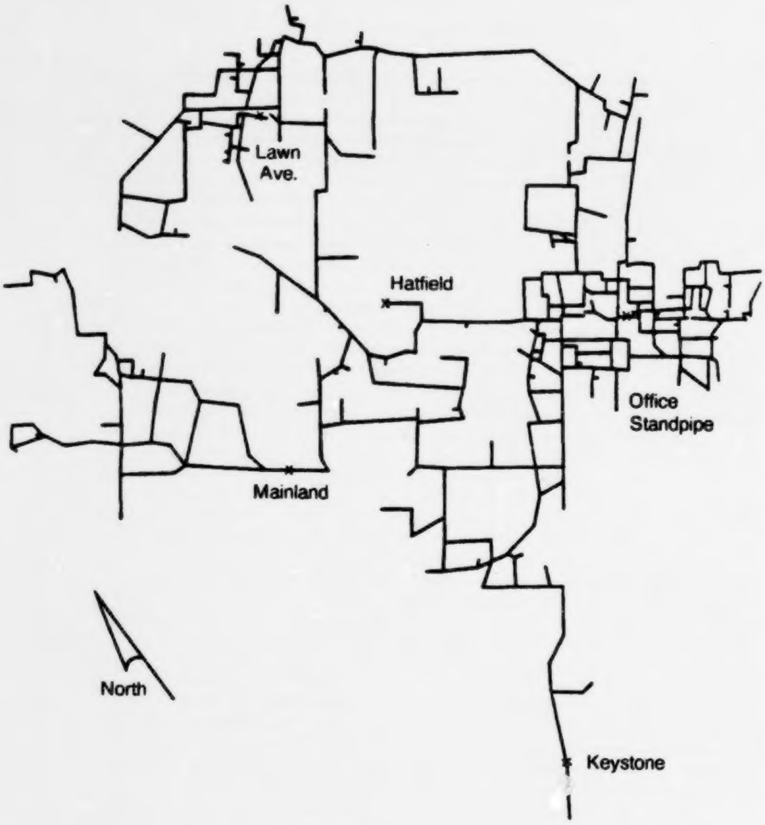


Figure 3.5.5. Sampling points for field study.

Sequential steady-state modeling

The Solver algorithm used to predict concentration is steady-state in nature, and relies upon boundary conditions and externally defined steady-state flows. The Solver algorithm was applied to a series of hydraulic scenarios, each representing a portion of the time period associated with the pilot sampling run. The pattern of flow in the Lansdale low zone was represented by three separate scenarios, each representing a portion of time during the day. For the calculations associated with the Solver predictions, the value of Keystone TTHM was set to 61 g/L, the average value encountered during the field testing. From 6:00 a.m. to noon, a scenario was selected in which a demand factor of 2 is applied, wells are on, and water is transferred

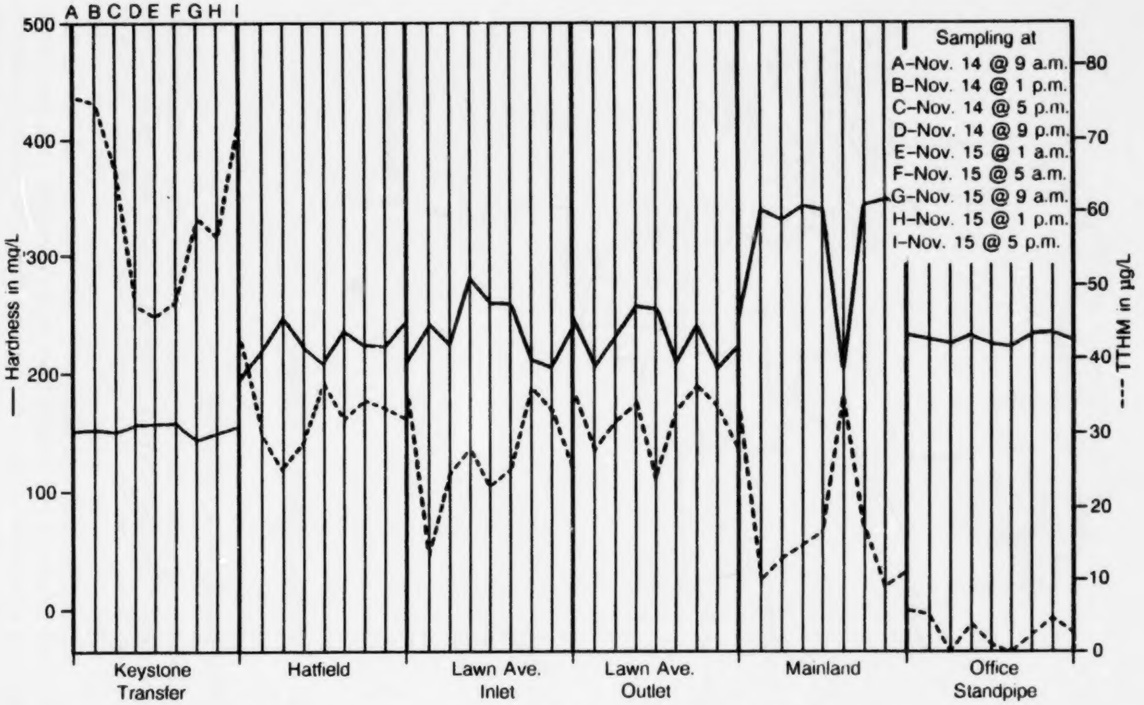


Figure 3.5.6. Sampling results from six sampling stations in North Penn Water Authority.

from the Hillcrest zone into Lansdale. Water flows out of the elevated tank, and into Lawn Avenue and the Chestnut Street standpipe. Figure 3.5.7 shows the predicted TTHM contours associated with this scenario. During this time period, with both wells on and a transfer from Hillcrest, the Keystone water is confined in the central portion of the pressure zone, and undergoes blending throughout this area. The Mainland sampling site is predicted to have 100 percent well water. The noon to midnight period was modeled using a scenario in which a demand factor of 1 is applied, wells are on, but there is no transfer of water from Hillcrest to Lansdale. Water flows into the Lawn Avenue, Chestnut Street, and elevated tanks. Figure 3.5.8 shows the TTHM contours associated with this period. Under this scenario, there is less flow from Keystone (demand is lower) than in the 6 a.m. to noon scenario, and there is greater flow of water into the tanks. Thus, there is a relatively greater amount of well water in the system, and the excursion of Keystone water northwards is somewhat suppressed, as can be seen by comparing Figures 3.5.7 and 3.5.8. The third period, from midnight to 6 a.m., is represented by a scenario for which wells are off, a demand factor of .5 is applied, and there is no Hillcrest water transferred to Lansdale. Under this scenario, all tanks are filling, and all water in the Lansdale low zone is derived from Keystone (all wells are off). The steady-state analysis thus dictates the following conclusions: the overall flow pattern is one of excursion of Keystone water northward in the system during the period of high demand (morning), some confinement of Keystone water in the afternoon, and then full excursion of Keystone water into the system at the period of low demand, when wells are off.

The actual THM measurements made during the pilot field study were presented in Figure 3.5.6. The most striking feature of this display is the dramatic change in the TTHM values associated with the Mainland sampling site, and, to a lesser degree, at the Lawn Avenue tank inlet. The water at Mainland has the lowest TTHM values at the 1:00 p.m. and 5:00 p.m. samples (points B and C, H and I), indicating the lowest percentage of Keystone water during that time period. This is consistent with the overall predicted pattern that suggests that Keystone water is most confined within the central portion of the system during the noon to midnight period. The water then travels throughout the system during the early morning hours when wells are off. The specific point prediction is for 100 percent well water at the Mainland sampling site during the two daytime prediction periods. The model does predict the flow reversals that do take place at the Mainland sampling site. The Lawn Avenue inlet site shows the lowest values of TTHM at the 1 a.m. sample on November 14 (point B on Figure 3.5.6). This is roughly consistent with the prediction of the largest confinement of Keystone water during the noon to midnight period. The relatively large change in TTHM from Keystone during the sampling period complicates the analysis, and further studies are planned, involving sampling for a longer period at shorter sampling

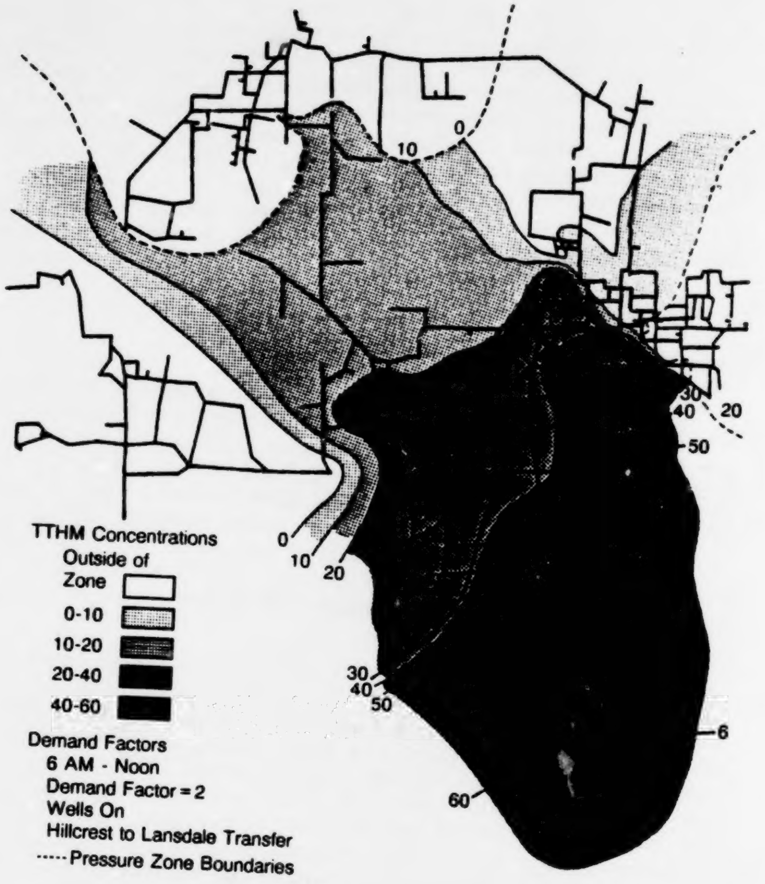

10
0
30
40
20
50
0
10
20
30
40
50
6
60
TTHM Concentrations
Outside of Zone ☐
0-10 ▨
10-20 ▨
20-40 ■
40-60 ■
Demand Factors
6 AM - Noon
Demand Factor = 2
Wells On
Hillcrest to Lansdale Transfer
····· Pressure Zone Boundaries

Figure 3.5.7. Predicted TTHM concentrations for demand factor = 2 and "wells on."

intervals, to better characterize the effects of the Keystone water for the analysis.

In general, the predicted distribution is consistent with the observed data, to the extent that general patterns of behavior are borne out. This technique is clearly useful for modeling and tracking the propagation of contaminants in distribution systems.

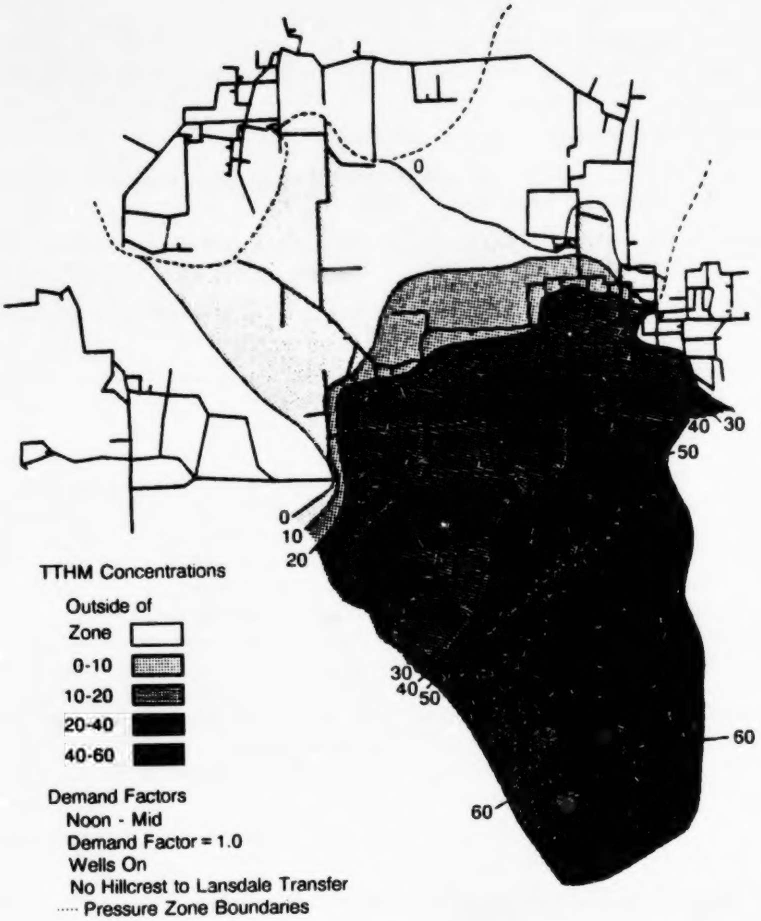

0
40
30
50
0
10
20
TTHM Concentrations
Outside of Zone
0-10
10-20
20-40
40-60
30
40
50
60
60
Demand Factors
Noon - Mid
Demand Factor = 1.0
Wells On
No Hillcrest to Lansdale Transfer
····· Pressure Zone Boundaries

Figure 3.5.8. Predicted TTHM concentrations for demand factor = 1.0 and "wells on."

Contaminant Propagation in a Distribution System

The work described previously demonstrates the feasibility of predicting general contaminant patterns in a distribution system. However, it is also useful to be able to trace water and contaminant movement in a system. Building on the sequential steady-state model, a microcomputer-based model has been developed for tracing the movement of water through a distribution system under steady-state conditions. As part of the water tracing model, the user specifies a source node for which analysis is to be performed. The model then traces flow from that node to all other nodes in the system and calculates:

- The total flow entering each node
- The fraction of flow entering each node that emanated from the designated source node
- The average travel time from the designated source node to every other node in the system
- The minimum travel time from the designated source node to every other node in the system
- The maximum travel time from the designated source node to every other node in the system
- A travel time divergence index

To perform the analysis, the model establishes a nonunique "hydraulic" ordering between all nodes and links in a distribution system. The ordering ensures that prior to operating on a given node, the flow from all links into that node have previously been analyzed. Prior to operating on a given link, flow from the upstream nodes will have been determined. Using the above ordering mechanism at each node, the total flow entering the node is calculated as the total flow entering from all incoming links plus the flow entering directly into the node if the node is a source. Similarly, the flow entering the node that originates from a designated source is calculated as the total flow in each link that emanated from this source. The fraction of flow entering the link emanating from the designated source is simply the ratio of the two values.

The average travel time from source node J to node I is calculated by the following formula:

$$TT_{IJ} = \{\Sigma[(TT_{kj} + TT_{KI}) \bullet F_{KJ} \bullet Q_{KI}]/(\Sigma F_{kj} \bullet Q_{KI})\} \qquad (6)$$

where

TT_{IJ} = travel time from source node J to node I

Σ = the summation over all directly upstream nodes (K)

TT_{KI} = travel time in the link from node K to node I

F_{kJ} = the fraction of flow at node K from source node J

Q_{KI} = flow in link from node K to node I

The minimum travel time from a source node to any node is the shortest time path between the nodes. This value is calculated by analyzing links and nodes using the previously described hydraulic ordering. At each node, the travel time is calculated for each entering link by adding the minimum travel time at the directly upstream node and the travel time in the link. The entering link with the minimum travel time is assigned to the node. A similar procedure is used to calculate the maximum travel time for each node.

A normalized travel time divergence index is calculated for each node using the following equation:

$$\text{Divergence Index} = (TT_{MAX} - TT_{MIN})/TT_{MIN} \qquad (7)$$

where TTMAX and TTMIN are the maximum and minimum travel times, respectively. This index is a measure of the divergence in travel times via alternative paths.

The water tracing model is designed for use in connection with a hydraulic model of a distribution system. The information required for the model corresponds to the network information used as input to a hydraulic model supplemented by flow information generated by the model. The input information required is listed in Table 3.5.2.

Table 3.5.2. Input Information Utilized by Water Tracing Model

- Source node to be analyzed
- Link Information
    - Link number
    - Upstream and downstream node number
    - Link length
    - Link diameter
    - Flow (quality and direction)
- Source node numbers and inflows

Application of Contaminant Propagation Model

The contaminant tracing model was applied to the North Penn distribution system under varying steady-state conditions reflective of the differing operating conditions and demands described earlier. In the first application, average demand was assumed and all wells were operating at the normal supply rate. Two sources were studied for this scenario: water supplied from the Keystone transfer (1,089 GPM) and water emanating from well NP14 (311 GPM). The pattern of flow emanating from the Keystone transfer is shown in Figure 3.5.9. This figure shows the fraction of flow reaching each area originating from Keystone. Figure 3.5.10 shows a similar pattern of flow from well NP14. As illustrated, both supplies serve as a significant source of water for the immediate area surrounding the respective source node and a less important source for parts of the system at a greater distance from the supply. Even though the two sources are located far apart, there are portions of the system that receive flow from both sources.

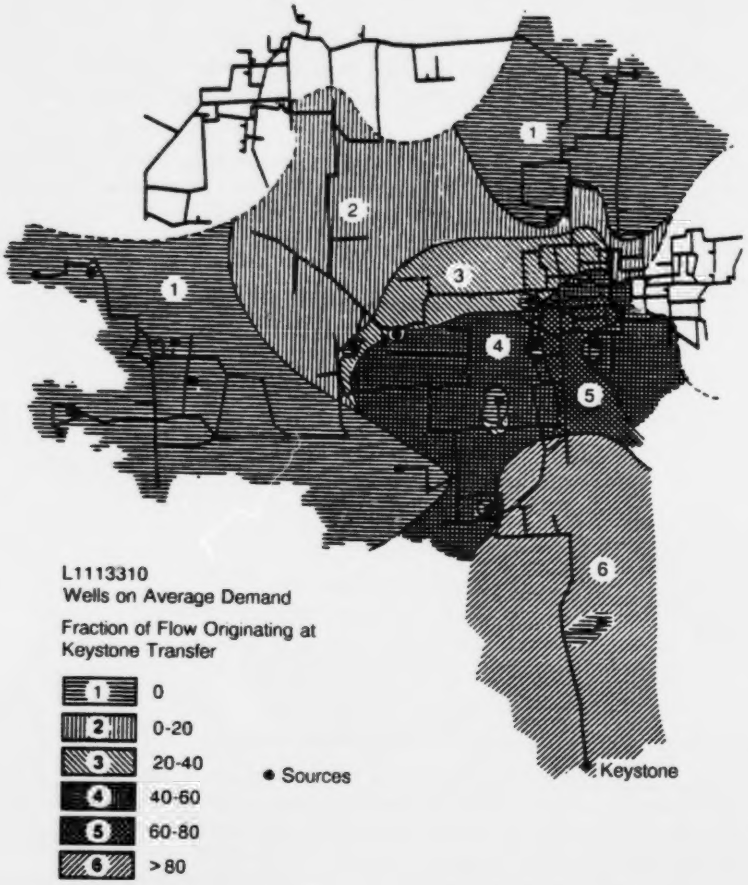

1
2
1
3
4
5
6
L1113310
Wells on Average Demand
Fraction of Flow Originating at Keystone Transfer
1 0
2 0-20
3 20-40
4 40-60
5 60-80
6 >80
• Sources
Keystone

Figure 3.5.9. Fraction of flow emanating from Keystone Transfer (average demand conditions and wells at normal supply rates).

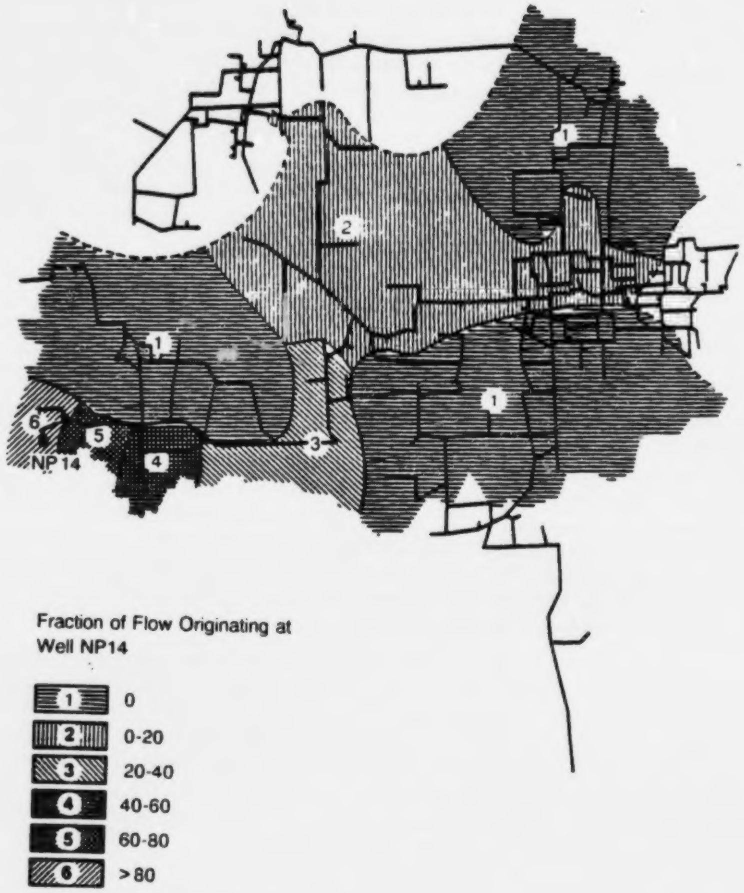

1
2
1
1
6
5
NP 14
4
3
Fraction of Flow Originating at Well NP14
1 0
2 0-20
3 20-40
4 40-60
5 60-80
6 >80

Figure 3.5.10. **Fraction of flow emanating from Well NP14 (average demand conditions and wells on normal supply rates).**

Travel Time

The average travel time from the two sources to other nodes served by the sources was calculated for the Keystone Transfer and well NP14, respectively. As would be expected, time of travel is approximately related to the distance from the source. In each of these scenarios, travel times to some small dead end pipes can be very long (i.e., several days) because the average demands at these points are very low. Figure 3.5.11 illustrates the calculation of the travel time diversity index for flow emanating from Keystone. A higher value of the diversity index indicates a greater normalized difference between the maximum and minimum travel time to that node. In general, a higher value results from multiple paths to a specific node. In Figure 3.5.11, a low diversity index (<0.1) is illustrated for a corridor connecting Keystone with the Lawn Avenue Tank in Souderton. This can be explained by the presence of only a single major transmission path between these two nodes. However, the diversity index is much higher (>5) in the vicinity of Lansdale, indicating a diversity of paths with significantly varying travel times.

Travel time plots were prepared for three other scenarios. A low demand scenario (half of average demand) with none of the wells operating showed that since Keystone is the only source during this scenario (all tanks, which can also be considered sources under steady-state conditions are filling in this scenario), the entire main pressure zone for the system receives flow from Keystone. Travel times in excess of 24 hours to some of the more distant parts of the system were found. High demand (twice average demand) and very high demand (four times average demand) scenarios were also prepared. In both cases all wells were operating. In the very high demand scenario, all three tanks were discharging so that Keystone water reached a smaller part of the overall system. When the travel times corresponding to the four scenarios are compared, the differences are relatively small and no significant association is apparent between travel time and demand.

In Figure 3.5.12, the travel time of water from the four main sources is plotted for the very high demand scenario. The four sources (Keystone plus the three tanks) deliver approximately two-thirds of the demand in this scenario. As illustrated, each of the sources supply water to the area surrounding the source and additionally overlap with other sources to supply areas more removed from the source. An extension of this work is described in the following section in the development of a dynamic water quality model.

Dynamic Water Quality Algorithm Development

The dynamic water quality model described in this paper uses a numerical routing solution to trace water quality through a distribution network (7). As described earlier, the distribution system is represented by a link-node system. Demands and inflows (both volumes and concentrations) are assumed constant over a user-defined period and a quasi-dynamic externally generated hydraulic solution is required for each period. Thus, the flow and velocity for each link is known from the hydraulic solution for each time period. Each time period is evenly divided into an integer number of computational time steps, Δt. Each link is then divided into sublinks by a series of evenly spaced subnodes (though the distance between subnodes may vary

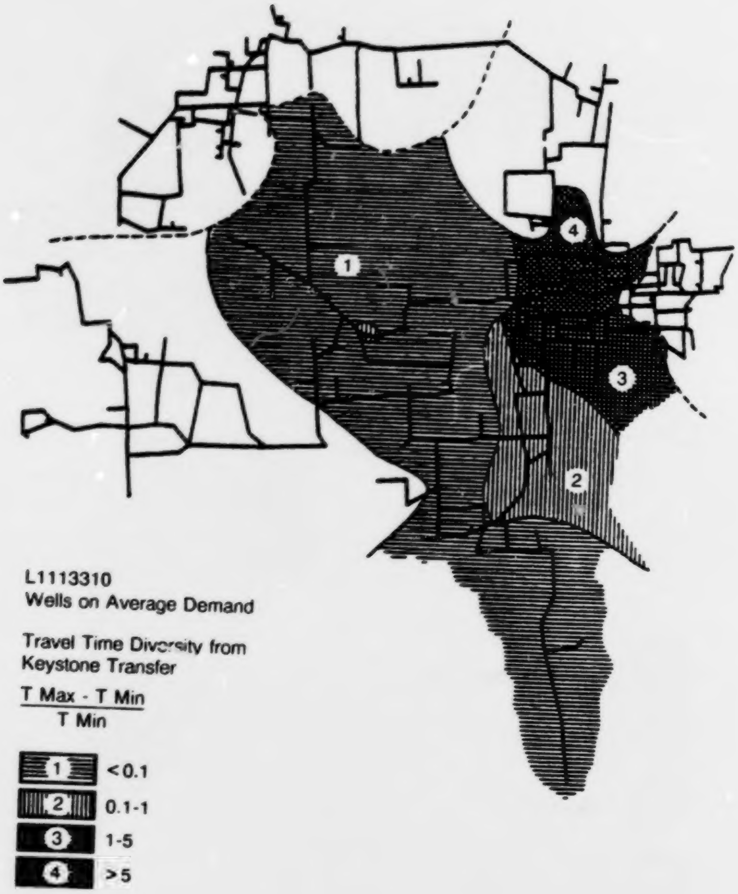

1
2
3
4
L1113310
Wells on Average Demand
Travel Time Diversity from Keystone Transfer
T Max - T Min / T Min
1 < 0.1
2 0.1-1
3 1-5
4 > 5

Figure 3.5.11. Travel time diversity from Keystone Transfer to various points in system (average demand conditions and wells on normal supply rates).

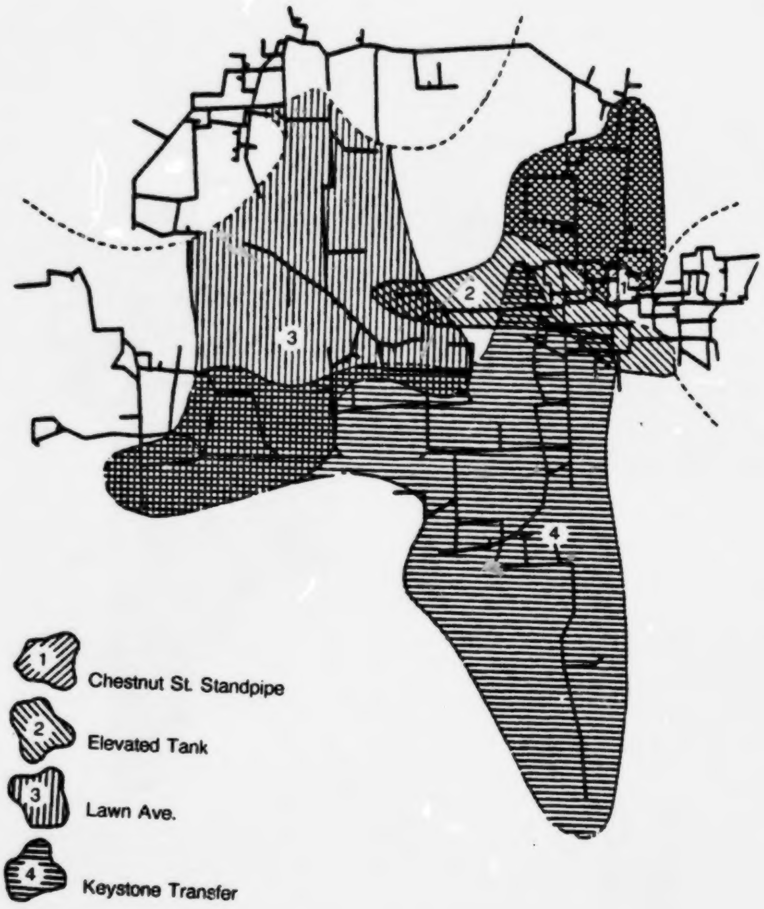

1
2
3
4
1 Chestnut St. Standpipe
2 Elevated Tank
3 Lawn Ave.
4 Keystone Transfer

Figure 3.5.12. Areas receiving flow from major sources and tanks.

from link to link or for a link at different time periods), such that the travel time from a subnode (or node) to the adjacent subnode (or node) is approximately equal to Δt.

Solution Procedure

The information required by the dynamic model may be classified into three categories: general information, initial conditions, and information required for each time period. Table 3.5.3 summarizes this required information.

Table 3.5.3. Information Required by the Dynamic Water Quality Model

---

General Information
- Dt-Time Step

General Network Information:

- Node numbers associated with the end of each Link
- Link lengths
- Pipe diameters
- Node number associated with each source
- Node number associated with each tank
- Tank geometry

Initial Conditions:

- Concentration at each node at the start of simulation
- Volume in tank at start of simulation

Information Required for Each Period:

- Direction and flow in each link
- Velocity in each link (optionally may be calculated based on pipe diameter)
- Concentration in source flow

The solution algorithm used in the dynamic water quality model operates sequentially by time period. During a time period, all external forces affecting the water quality are assumed to remain constant (i.e., demand, well pumpage, tank head, etc.).

An example of the movement of a contaminant is illustrated in Figure 3.5.13. In this example, the relative concentration within each subnode is illustrated by the depth of shading with black corresponding to high concentration and white corresponding to low or zero concentration. As illustrated, during each time step, the contents of a subnode moves to the adjacent subnode traveling in the direction of flow. In this example, at the end of a time period (i.e., after time step T3), the direction of flow changes and the number of subnodes change due to an increase in velocity. Accordingly, the high concentration packets shift direction and move from right to left.

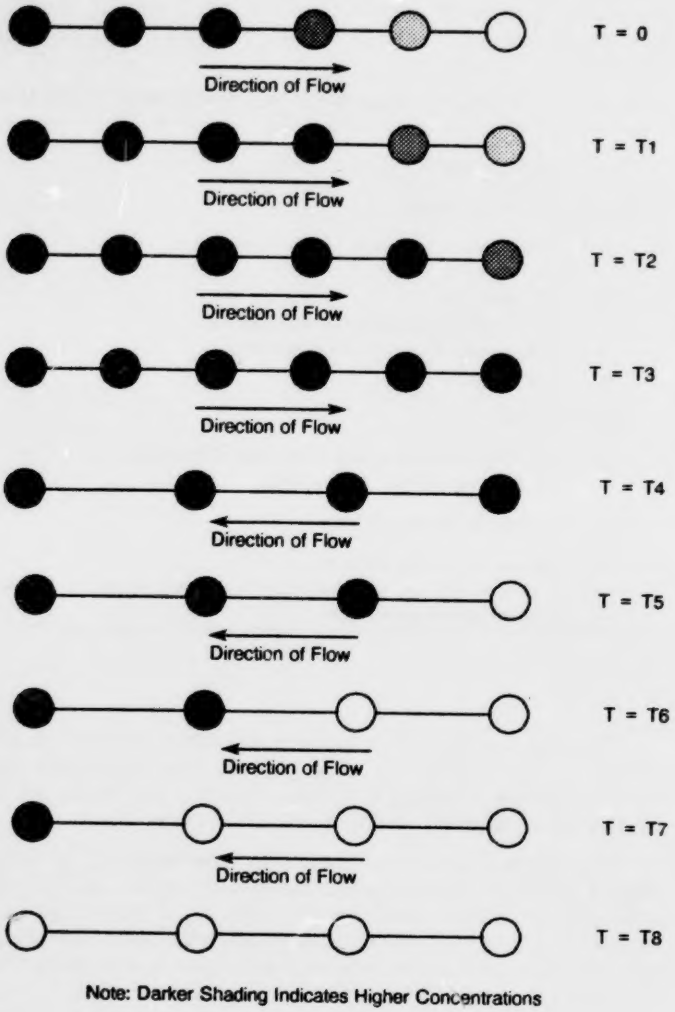

T = 0
Direction of Flow
T = T1
Direction of Flow
T = T2
Direction of Flow
T = T3
Direction of Flow
T = T4
Direction of Flow
T = T5
Direction of Flow
T = T6
Direction of Flow
T = T7
Direction of Flow
T = T8
Note: Darker Shading Indicates Higher Concentrations

Figure 3.5.13. Example movement of contaminant within link.

Model Application

The dynamic water quality algorithm was implemented as a microcomputer-based dynamic water quality model written in FORTRAN. Researchers developed versions for both the Apple Macintosh computer and IBM-PC (or compatible) computer and applied the model in a full-scale demonstration on the NPWA distribution system (8).

The dynamic water quality model was used to model the movement of constituents in the North Penn system, during a 34-hour period of simulated conditions corresponding to those present during the pilot level sampling program conducted on November 14-15, 1985. Hydraulic conditions in the system were determined using the WADISO hydraulic model. Researchers adjusted model parameters so that predicted tank levels and flows at selected sites represented those measured during the sampling period. Figure 3.5.14 compares measured and modeled hydraulic conditions at three locations.

Flows in the network were aggregated into seven periods, ranging in length from 2 to 7 hours, during which flows throughout the system remained relatively constant. These flows were input into the dynamic water quality model along with information on initial tank water levels.

Three water quality constituents were modeled: TTHM, chloroform, and hardness. Chloroform is present only in the Keystone water source. Another component of TTHM, bromoform, is formed in the system from precursors present in many of the wells. Based on sampling results, a constant chloroform concentration of 38 µg/L was introduced into the model at the Keystone import. Similarly, initial concentrations of chloroform of 14 µg/L, 1 µg/L, and 1 µg/L in the Lawn Avenue tank, elevated tank, and Chestnut Street standpipe, respectively were used based on the field sampling results. The dynamic water quality model was initially applied for a 24-hour period and the resulting nodal concentrations used as initial conditions for the 34-hour model application. This means of estimating initial conditions is based on the assumption that the model will reach a nonsteady-state equilibrium within the 24 hours and that the system displays an approximate 24-hour periodic cycle. Examination of modeling results and results of the field sampling program validated both assumptions. For all runs of the dynamic water quality model, chloroform was treated as conservative (i.e., no chemical formation of chloroform was assumed) and a time step of 30 minutes was used. The resulting predicted time history of chloroform is plotted in Figure 3.5.15, along with field sampling results for three sampling stations.

In modeling TTHM, concentration was set at 60 µg/L at Keystone, the average concentration observed during the sampling program. Concentrations of TTHM (corresponding to the formation of

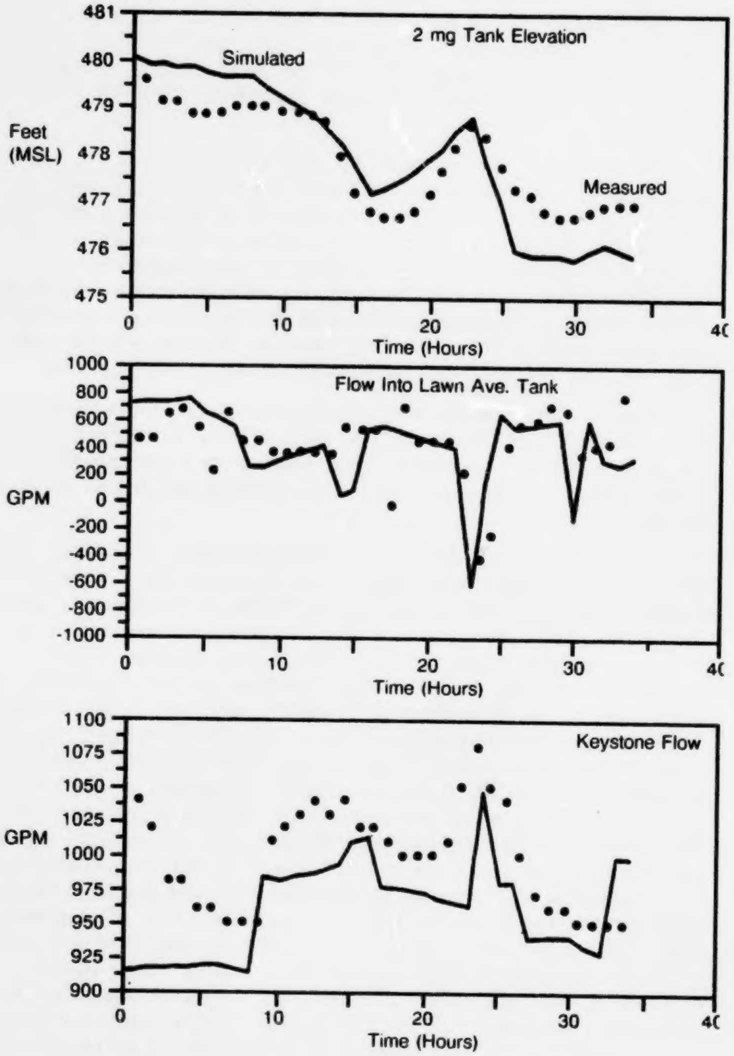


Figure 3.5.14. Comparison of measured and simulated hydraulic conditions.

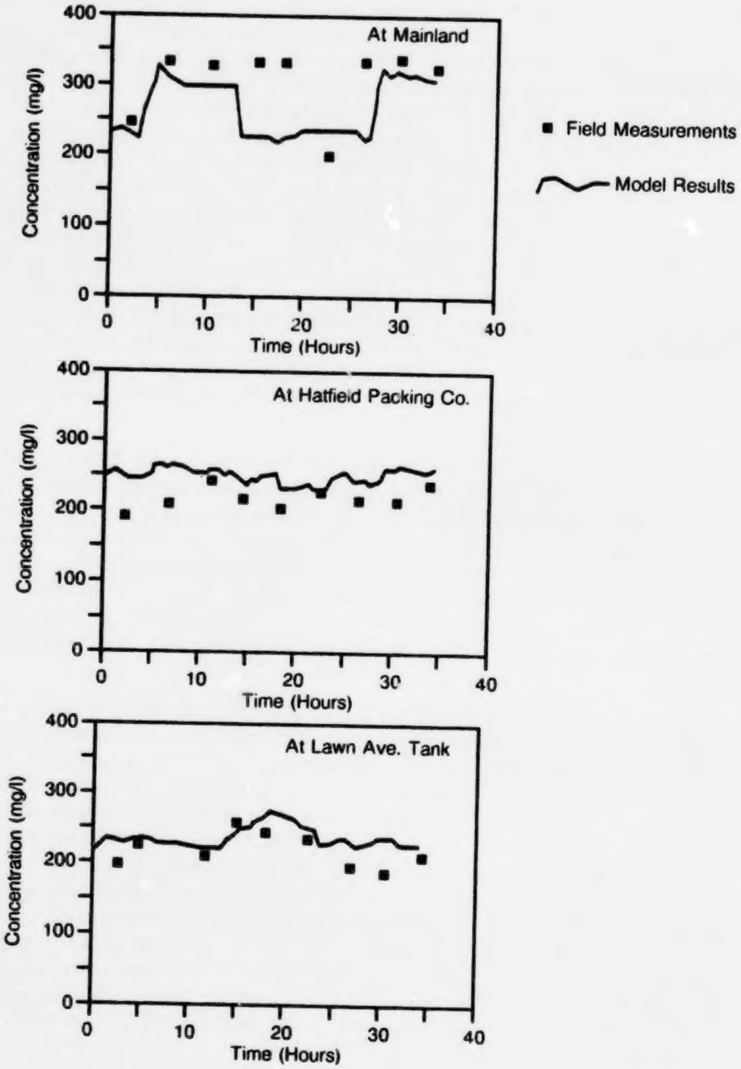


Figure 3.5.15. Comparison of modeled and measured hardness a selected stations.

bromoform) were assumed to be 10 µg/L at all wells. This value is purely an assumption since water quality was not measured at the wells during the sampling period. Additionally, historical measurements of TTHM or bromoform were not available because only the precursors of bromoform are present in the wells rather than bromoform itself. Initial concentrations of TTHM of 34 µg/L at Lawn Avenue Tank, and 5 µg/L at the elevated tank and Chestnut Street standpipe were used based on the results of the field sampling program. Modeling results for TTHM are plotted in Figure 3.5.16 for three sampling stations along with the sampling results at those locations. The spatial variation of TTHM, as predicted by the model, are plotted in Figure 3.5.17 for hour 9 (9 hours after the start of the simulation) and in Figure 3.5.18 for hour 24. Hour 9 corresponds to a period of high demand when most wells are operating and the spatial influence of Keystone water is at a minimum. Hour 24, at 7:00 a.m., corresponds to the end of a low demand/low well pumpage period; a time at which the Keystone water has traveled to its greatest extent through the system.

The hardness of the water sources for the North Penn system varies considerably. During the sampling period average hardness for the Keystone water was 318 mg/L. No measurements of hardness were taken at the wells during the sampling period, but based on routine sampling during the months previous to and following the sampling period, average hardness for the wells varied from 179 to 413. The results of the predicted hardness as compared to sampling measurements are presented in Figure 3.5.15 for the three sampling stations.

Examination of the results of the application of the dynamic water quality model to the North Penn system as reflected in Figures 3.5.15 to 3.5.19 indicate both close agreement between predicted and observed results and some anomalous behavior. For chloroform, TTHM, and hardness the general levels of concentration compare very favorably to the observed values at the three selected sampling stations (Figures 3.5.16 and 3.5.17). In each case, there are some differences in the timing of peak or minimum values. When the spatial variation of predicted TTHM concentrations are compared to the historical average TTHM level, the same general patterns are apparent. Additionally, the predicted patterns bracket the pattern corresponding to the long-term historical average; a result that would be expected since the two selected times correspond to the extreme spatial patterns during the sampling period.

There are several known factors which could contribute to the variation between predicted and observed values. These factors are summarized below:

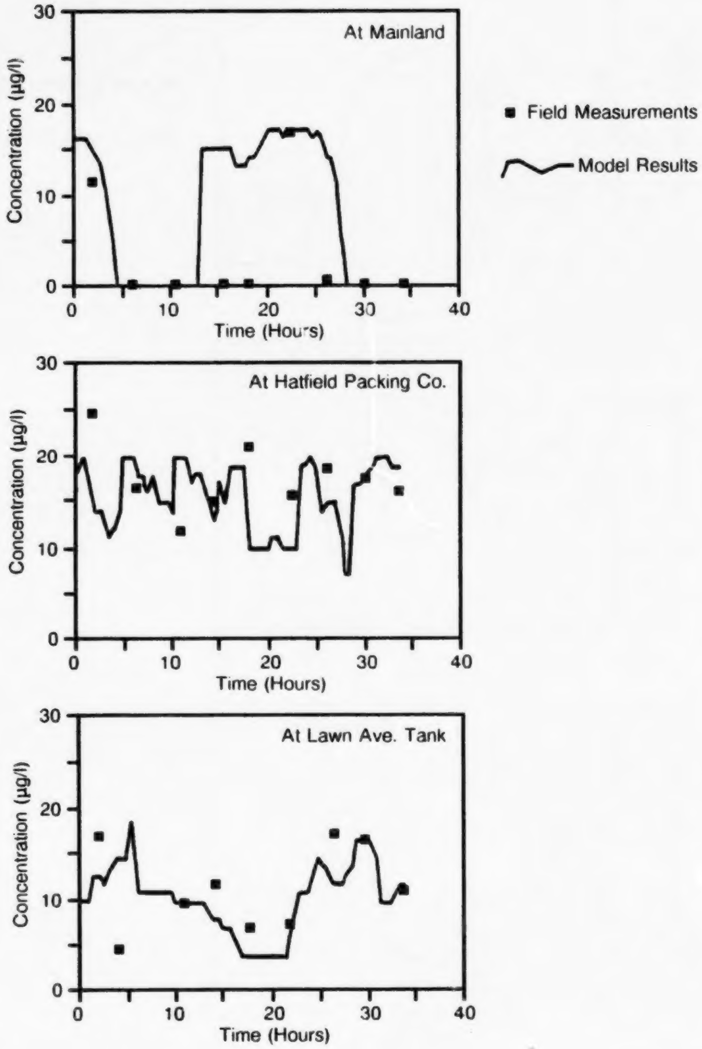


Figure 3.5.16. **Comparison of modeled and measured chloroform at selected stations.**

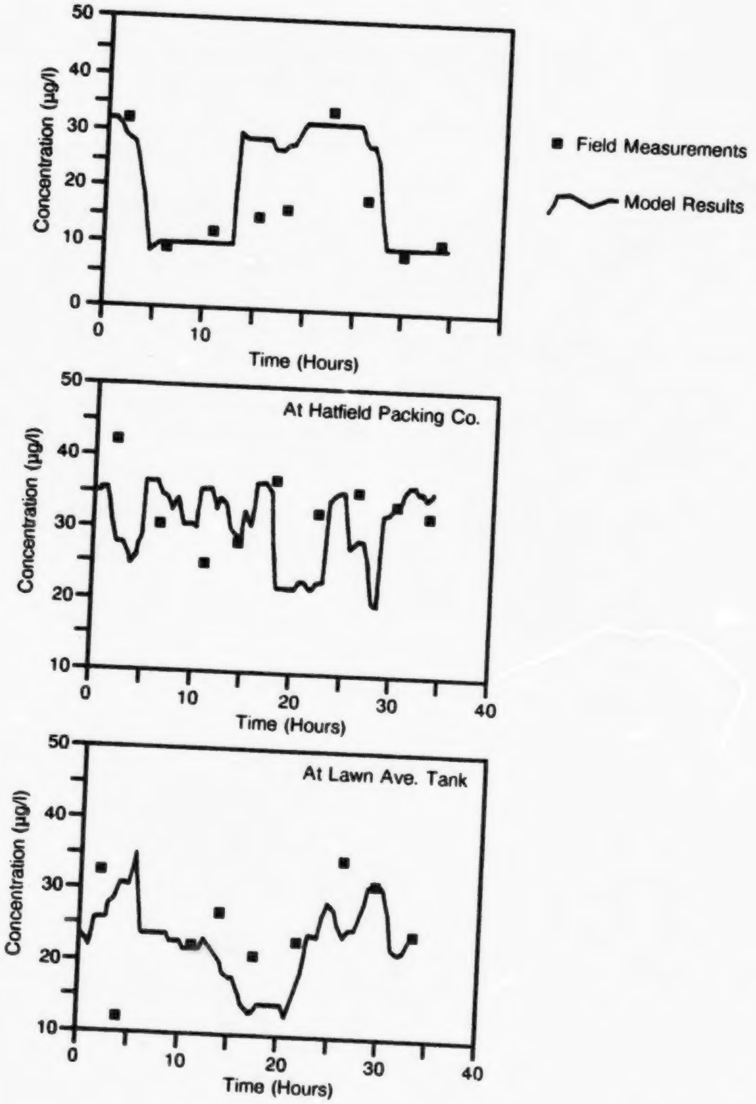


Figure 3.5.17. Comparison of modeled and measured TTHM at selected stations.

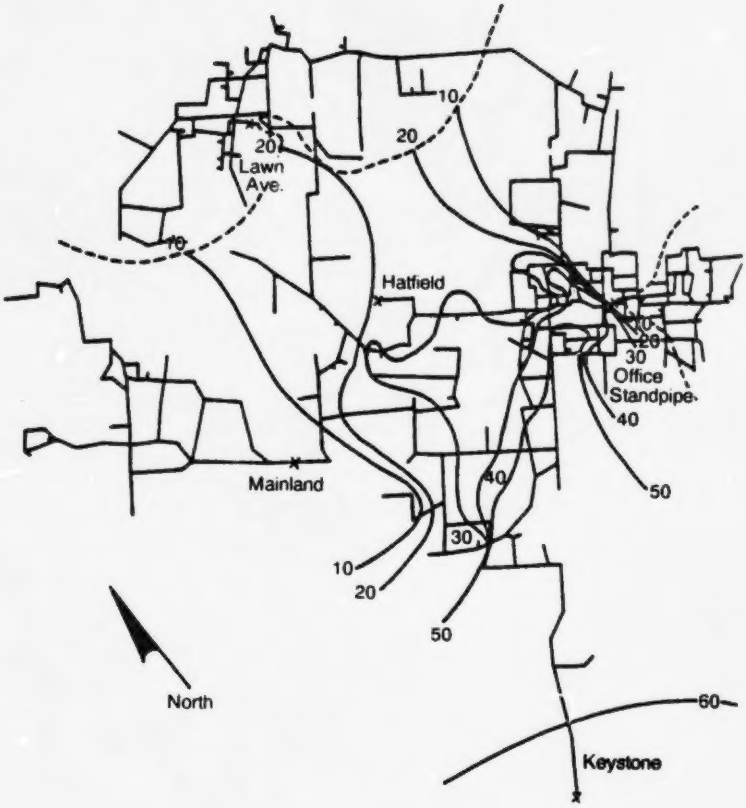

10
20
20
Lawn
Ave.
10
Hatfield
10
20
30
Office
Standpipe
40
50
Mainland
40
30
10
20
50
North
60
Keystone

Figure 3.5.18. Simulation of TTHM values in North Penn distribution network (Hour 9).

1. Observed temporal variations in the concentration of TTHM, chloroform, and hardness at Keystone were not represented in the simulation.
2. Field data on TTHM concentrations at the wells were not available and thus assumed values were not based on observed values.
3. Field data on hardness in well water were not available during the sampling period so that representative values based on sampling at other times were used.

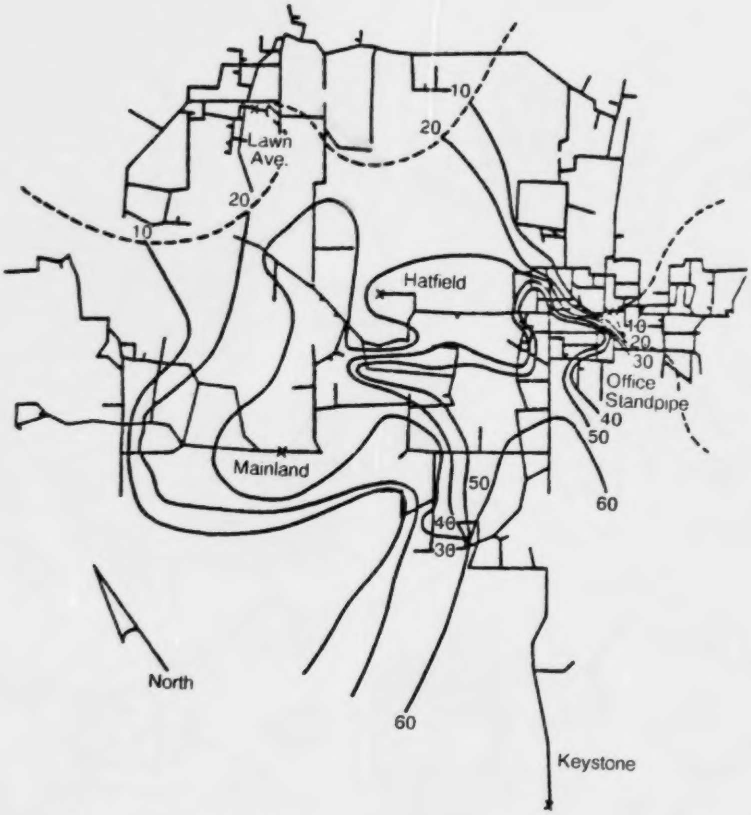

Lawn Ave.
Hatfield
Mainland
Office Standpipe
Keystone
North
10
20
20
10
10
20
30
40
50
50
40
30
60
60

Figure 3.5.19. Simulation of TTHM values in North Penn distribution network (Hour 24).

4. The nonconservative aspects of TTHM and chloroform were not represented in the model application.
5. Flows from the hydraulic model were aggregated into periods of various lengths thus losing some accuracy in representing high frequency hydraulic occurrences in the model.

Dynamic Model and Flow Tracing

The dynamic model was combined with the flow tracing to predict the movement of contaminants over time in the North Penn system. Figure 3.5.13 illustrates that the contaminant can move back and forth in the pipes under varying demand and hydraulic scenarios. In order to extend the contaminant propagation modeling capability, the tracking model was coupled with the dynamic hydraulic scenarios to predict contaminant movement.

To illustrate this effect, an initial influent of 100 units of contaminant was assumed injected at the Keystone tie-in at time equal to zero. Assuming time periods that were representative of a typical day (Figures 3.5.20a, b, c, and 3.5.21a, b, c, d), the contaminant was tracked in the North Penn system. Figures 3.5.20a, b, and c show the dispersion of the contaminant at t = 300 min. As shown, the contaminant is localized in the southeastern portion of the system. At t = 540 min, the contaminant has spread to the central portion of the system. By t = 960 min, the contaminant is beginning to disperse. At times l,440 min and 1,560 min, the contaminant is well dispersed and is beginning to disappear. By t = 2,040 min (nearly a day and a half),the contaminant still persists at very low levels, but is virtually gone from the system.

To more clearly illustrate this effect, a time trace of contaminant movement was calculated at three points in the system. These points are shown in Figure 3.5.22. Figure 3.5.23 shows the predicted time trace of the contaminant at points 3, 240, and 276. Point 3 shows the contaminant moving past the monitoring point into the system. Point 240 is a point of maximum mixing in the system in which water tends to move back and forth. Point 276 also illustrates the retention of contaminant in the pipe as water moves back and forth past a given point.

Discussion

Based on the results to date, steady-state predictive modeling appears to be a reasonable first step to characterize the distribution of water quality in multi-source systems. Although point prediction capability is probably not accurate, and is likely highly sensitive to hydraulic assumptions, general trends can be established and then verified through field sampling.

Steady-state prediction might be better in systems that are less dynamic in terms of operation, with fewer sources, than the NPWA distribution system. Steady-state assumptions also might be more consistent with a less dynamic system. In addition, the NPWA system is somewhat "disjointed," due to the manner in which it was developed, through connection of a number of separate systems. Thus a number of portions of the distribution system are connected by a single pipe. Once the hydraulic solution establishes flow direction, the system is effectively "partitioned," at least in certain areas, into zones of uniform concentration.

The steady-state algorithm as applied in this case did not take into account the concentration of Keystone water resident in the tanks.

Node with C > 10 based on injection of spike of C = 1000 at NP34 during period 1

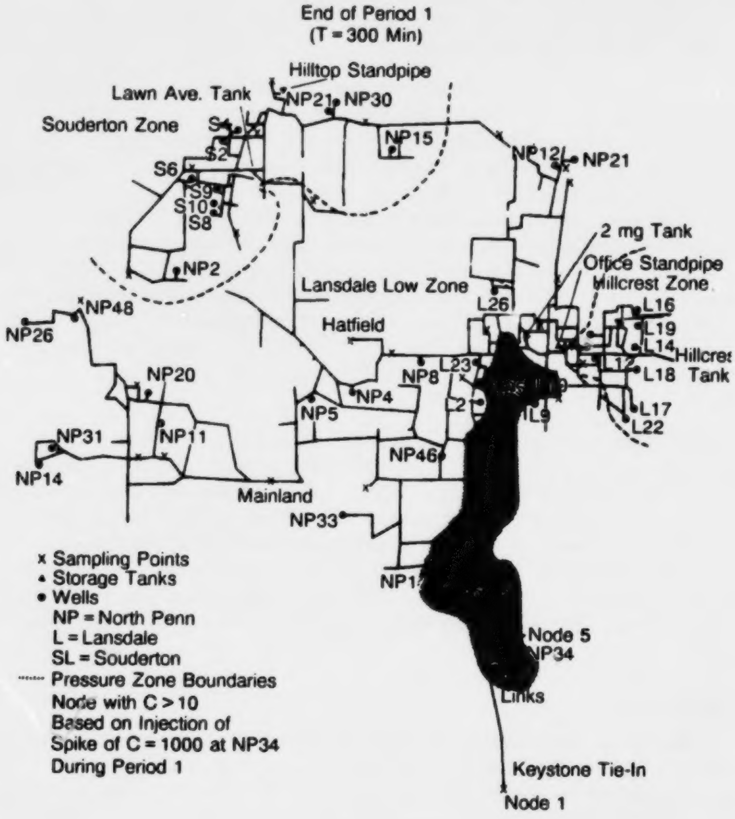

End of Period 1
(T = 300 Min)
Hilltop Standpipe
Lawn Ave. Tank
NP21
NP30
Souderton Zone
S4
S2
S6
S9
S10
S8
NP15
NP12
NP21
2 mg Tank
Office Standpipe
Hillcrest Zone
NP2
Lansdale Low Zone
L26
L16
L19
L14
Hillcres
Tank
L12
L18
NP48
NP26
Hatfield
NP8
L23
L2
L9
L17
L22
NP20
NP4
NP5
NP31
NP11
NP46
NP14
Mainland
NP33
NP1
Node 5
NP34
Links
Keystone Tie-In
Node 1
x Sampling Points
▴ Storage Tanks
• Wells
NP = North Penn
L = Lansdale
SL = Souderton
Pressure Zone Boundaries
Node with C > 10
Based on Injection of
Spike of C = 1000 at NP34
During Period 1

Figure 3.5.20a. **Simulated contaminant dispersion in North Penn system.**

According to the "late night" scenario (in which wells are off), at least some Keystone water will enter the tanks, and then be discharged into the distribution system as another "source" of Keystone water. This is a more complex analysis, but, in the majority of the hydraulic scenarios selected in this study, tanks were filling, rather than discharging to the system.

A less tractable problem is the issue of residence time of water in the pipes. The steady-state analysis fails to take into account water already in the pipes of the distribution system. For example, the late night scenario results in Keystone water flowing throughout the system, for all demands. In fact, much of the water in the pipe is well

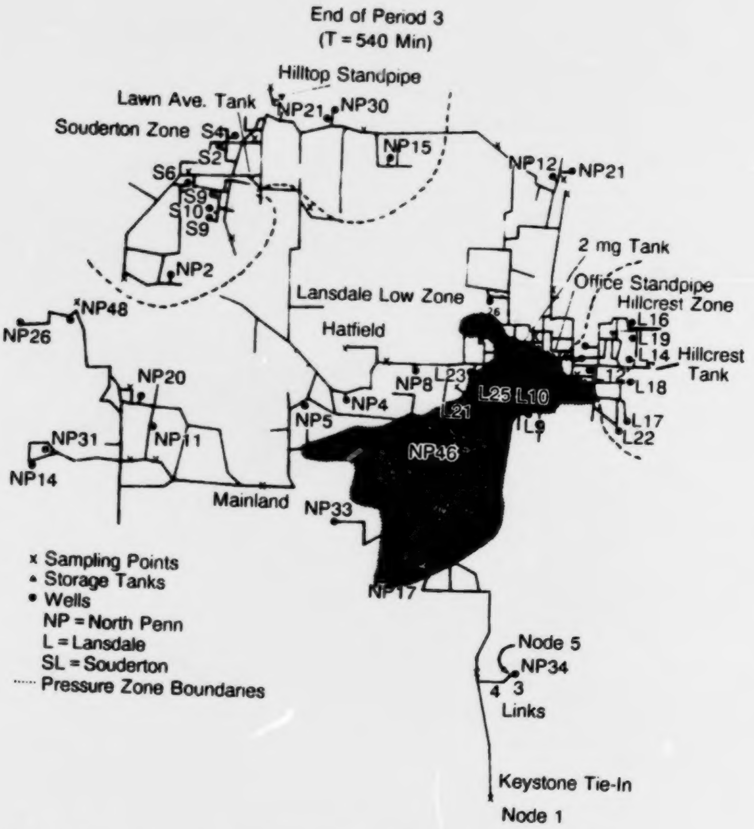

End of Period 3
(T = 540 Min)
Hilltop Standpipe
Lawn Ave. Tank
NP21
NP30
Souderton Zone
S4
S2
S6
S9
S10
S9
NP15
NP12
NP21
2 mg Tank
Office Standpipe
Hillcrest Zone
L16
L19
L14
Hillcrest Tank
L18
L17
L22
NP2
NP48
NP26
Lansdale Low Zone
Hatfield
NP8
L23
L25
L10
L21
L9
NP4
NP5
NP46
NP20
NP31
NP11
NP14
Mainland
NP33
NP17
Node 5
NP34
4
3
Links
Keystone Tie-In
Node 1
× Sampling Points
▲ Storage Tanks
● Wells
NP = North Penn
L = Lansdale
SL = Souderton
······ Pressure Zone Boundaries

Figure 3.5.20b. **Simulated contaminant dispersion in North Penn system.**

water, and the pipes act as a reservoir of this water, even though wells are off. Obviously, blending takes place, as is seen at the Mainland sampling site, rather than water being either fully well water or fully Keystone water. The volume of water in the pipes of the distribution system (as represented in the water supply simulation model, i.e., neglecting smaller pipes) was calculated to be 2.8 million gallons, or over half of the average daily supply of 5 million gallons for the NPWA distribution system. Thus, the reservoir effect is likely to be significant and cannot be taken into account in traditional steady-state modeling.

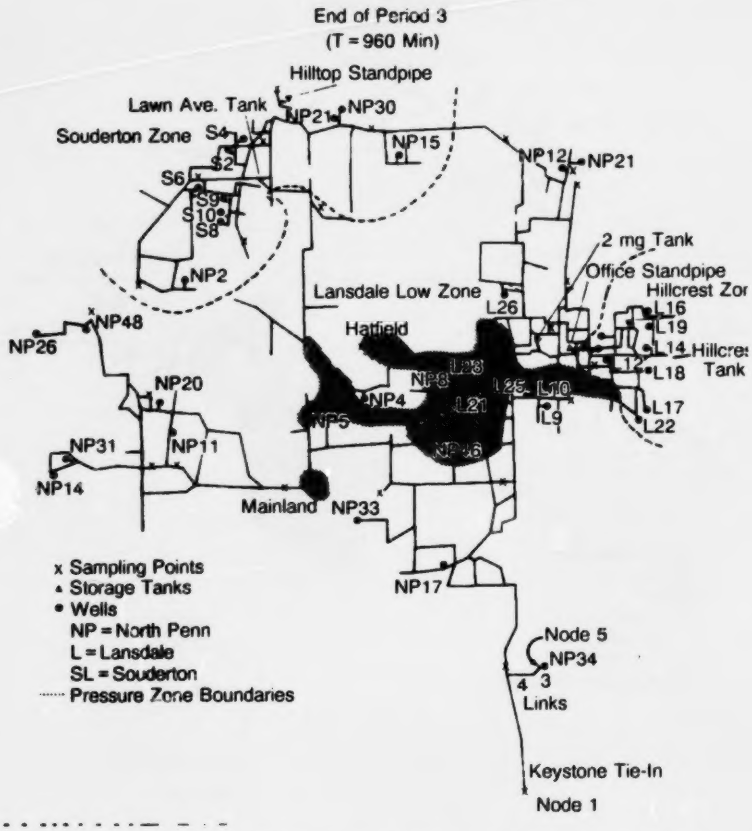

End of Period 3
(T = 960 Min)
Hilltop Standpipe
Lawn Ave. Tank
NP21
NP30
Souderton Zone
S4
S2
S6
S9
S10
S8
NP15
NP12
NP21
NP2
2 mg Tank
Office Standpipe
Hillcrest Zor
Lansdale Low Zone
L26
L16
L19
L14
Hillcre
Tank
L18
L17
L22
NP48
NP26
Hatfield
L28
NP8
L25
L10
L9
L21
NP4
NP5
NP16
NP20
NP31
NP11
NP14
Mainland
NP33
NP17
x Sampling Points
▲ Storage Tanks
● Wells
NP = North Penn
L = Lansdale
SL = Souderton
······ Pressure Zone Boundaries
Node 5
NP34
4
3
Links
Keystone Tie-In
Node 1

Figure 3.5.20c. **Simulated contaminant dispersion in North Penn system.**

This research includes extending the steady-state modeling approach to the prediction of time of travel from one point in the distribution system to another, and to calculating the percentage of water that supplies a given point in the system from all other sources. Both of these calculations have implications with regard to water quality and propagation of contaminants within the system. Development of a dynamic water quality model provides insight into the actual operation of the system and more accurately models the interaction of water quality and hydraulic behavior.

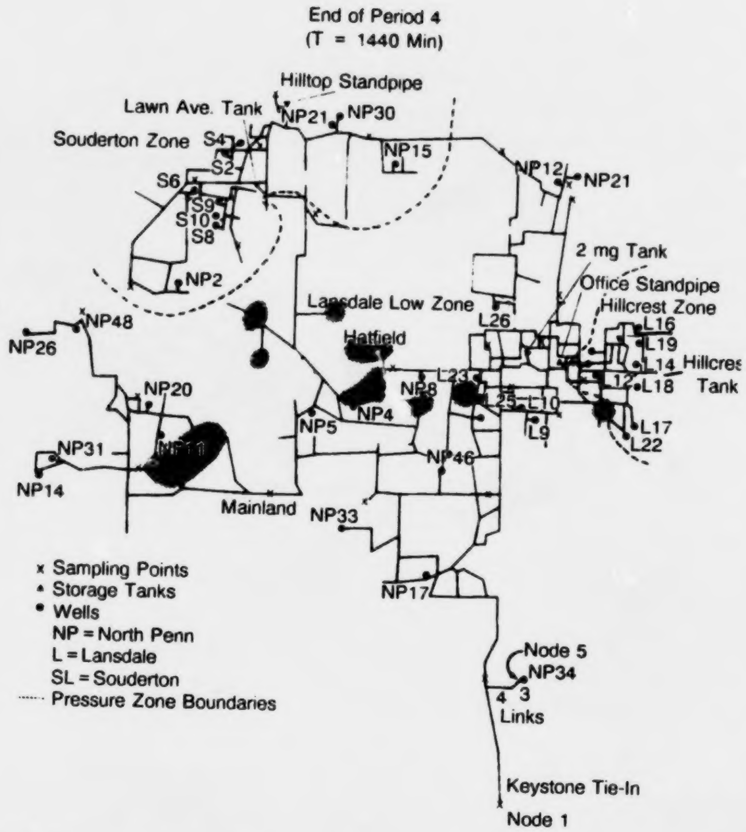

End of Period 4
(T = 1440 Min)
Hilltop Standpipe
Lawn Ave. Tank
NP21
NP30
Souderton Zone
S4
S2
S6
S9
S10
S8
NP15
NP12
NP21
2 mg Tank
Office Standpipe
Hillcrest Zone
NP2
NP48
NP26
Lansdale Low Zone
L26
Hatfield
L16
L19
L14
Hillcres
Tank
L12
L18
L23
NP8
L25
L10
L9
NP20
NP4
NP5
L17
L22
NP31
NP11
NP46
NP14
Mainland
NP33
× Sampling Points
▴ Storage Tanks
● Wells
NP = North Penn
L = Lansdale
SL = Souderton
······ Pressure Zone Boundaries
NP17
Node 5
NP34
4 3
Links
Keystone Tie-In
Node 1

Figure 3.5.21a. **Simulated contaminant dispersion in North Penn system.**

This research clearly indicates the need to obtain more representative monitoring results than are normally acquired from distribution system sampling. As can be seen from Figure 3.5.6, contaminant values can vary greatly over a relatively short time at a given point. There are also no doubt weekly and yearly cycles which, when combined with hydraulic and mixing variations, will have great effect on the contaminant levels at a given point in a distribution system. To provide insight into these variations, several automated samplers are being tested for installation in the North Penn system. Currently a rotary sampler fitted with an EPA-designed capping

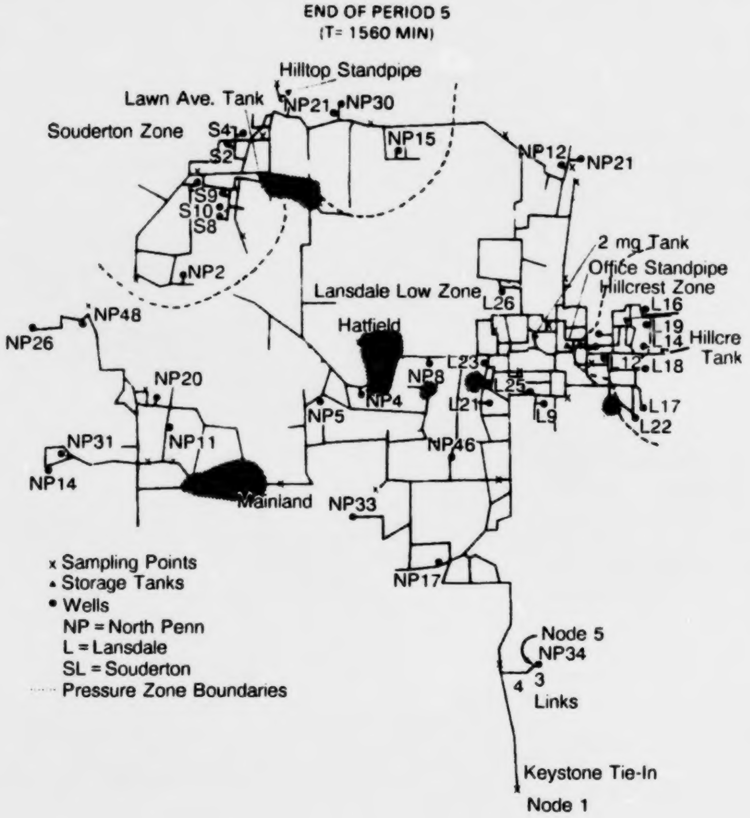

END OF PERIOD 5
(T= 1560 MIN)
Hilltop Standpipe
Lawn Ave. Tank
NP21
NP30
Souderton Zone
S4
S2
NP15
NP12
NP21
S9
S10
S8
2 mg Tank
Office Standpipe
Hillcrest Zone
NP2
Lansdale Low Zone
L26
L16
NP48
Hatfield
L19
L14
Hillcre Tank
NP26
L23
L12
L18
NP20
NP8
NP4
L25
L21
L17
NP5
L9
L22
NP31
NP11
NP46
NP14
Mainland
NP33
x Sampling Points
▲ Storage Tanks
● Wells
NP = North Penn
L = Lansdale
SL = Souderton
Pressure Zone Boundaries
NP17
Node 5
NP34
4
3
Links
Keystone Tie-In
Node 1

Figure 3.5.21b. **Simulated contaminant dispersion in North Penn system.**

system is being field tested. This sampler will allow samples to be taken at specified intervals for several days. The approach appears promising.

Conclusions and Recommendations

Steady-state predictive modeling of water quality can provide insight into overall water quality variations and patterns within a distribution system, although "point prediction" of water quality is less feasible. Interpretation of predictive modeling results must be

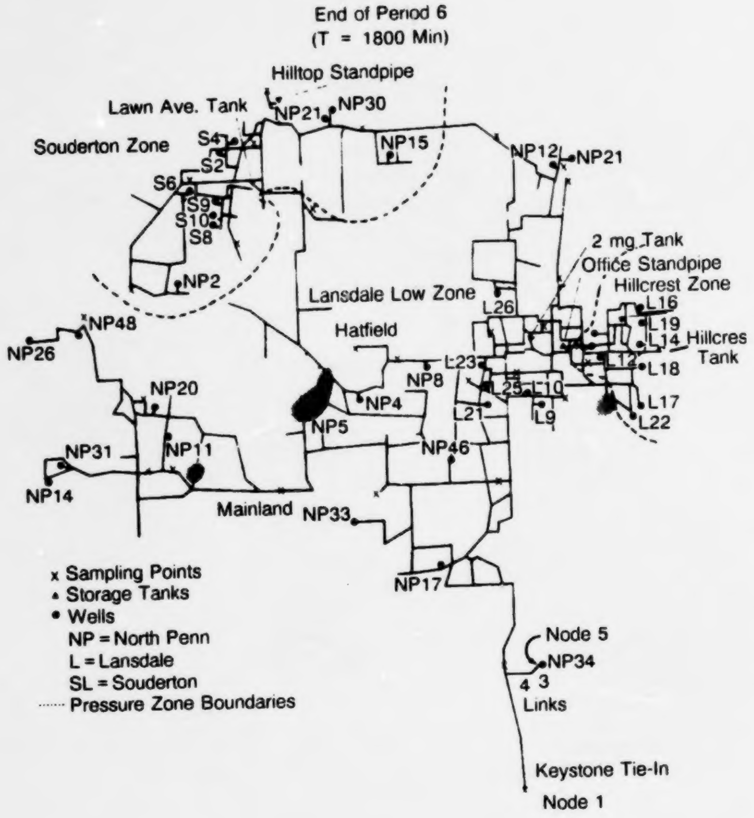

End of Period 6
(T = 1800 Min)
Hilltop Standpipe
Lawn Ave. Tank
NP21
NP30
Souderton Zone
S4
S2
S6
S9
S10
S8
NP15
NP12
NP21
NP2
2 mg. Tank
Office Standpipe
Hillcrest Zone
Lansdale Low Zone
L26
L16
L19
L14
Hillcres Tank
NP48
NP26
Hatfield
L23
L12
L18
NP8
L25
L10
NP20
NP4
L21
L9
L17
L22
NP5
NP31
NP11
NP46
NP14
Mainland
NP33
NP17
x Sampling Points
▲ Storage Tanks
• Wells
NP = North Penn
L = Lansdale
SL = Souderton
······ Pressure Zone Boundaries
Node 5
NP34
4 3
Links
Keystone Tie-In
Node 1

Figure 3.5.21c. Simulated contaminant dispersion in North Penn system.

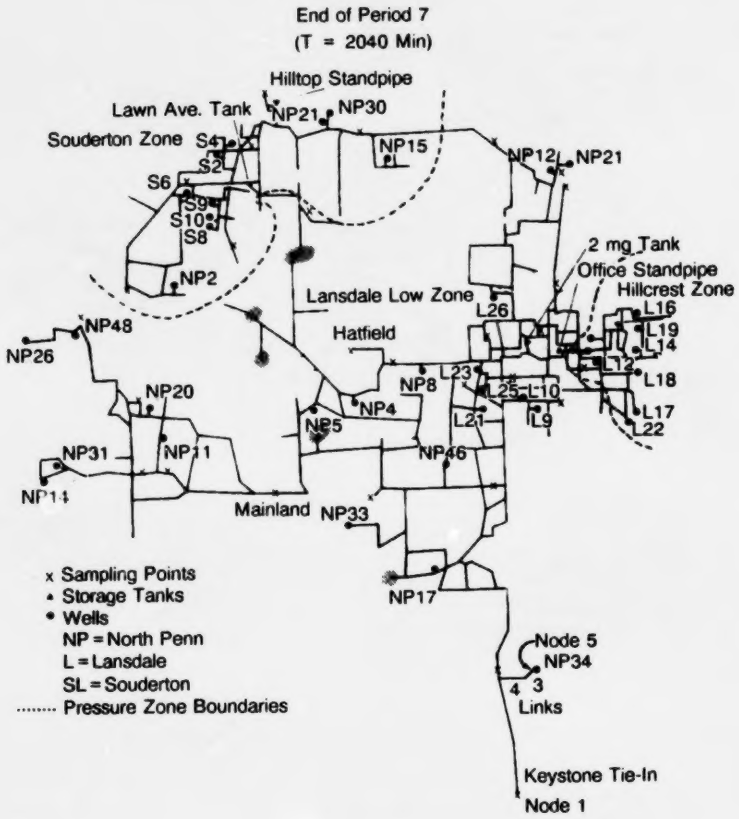

End of Period 7
(T = 2040 Min)
Hilltop Standpipe
Lawn Ave. Tank
NP21
NP30
Souderton Zone
S4
S2
NP15
NP12
NP21
S6
S9
S10
S8
NP2
2 mg Tank
Office Standpipe
Hillcrest Zone
Lansdale Low Zone
L26
L16
L19
L14
NP48
NP26
Hatfield
L12
L23
NP8
L18
NP20
L25
L10
NP4
L17
L22
L21
L9
NP5
NP31
NP11
NP46
NP14
Mainland
NP33
x Sampling Points
• Storage Tanks
• Wells
NP = North Penn
L = Lansdale
SL = Souderton
.... Pressure Zone Boundaries
NP17
Node 5
NP34
4
3
Links
Keystone Tie-In
Node 1

Figure 3.5.21d. **Simulated contaminant dispersion in North Penn system.**

made in light of an appreciation of the hydraulics of the system, in particular, an understanding of the flow patterns and directions that create the gradients of concentration. Quality modeling is based on hydraulic modeling, and is thus highly sensitive to hydraulic modeling assumptions and results. Field quality data are important in developing, verifying, and understanding predictive models. Such quality data should be available at time intervals sufficient to reflect daily changes in system dynamics. Having the tools to predict time of travel between points in a system and to estimate the quality of water

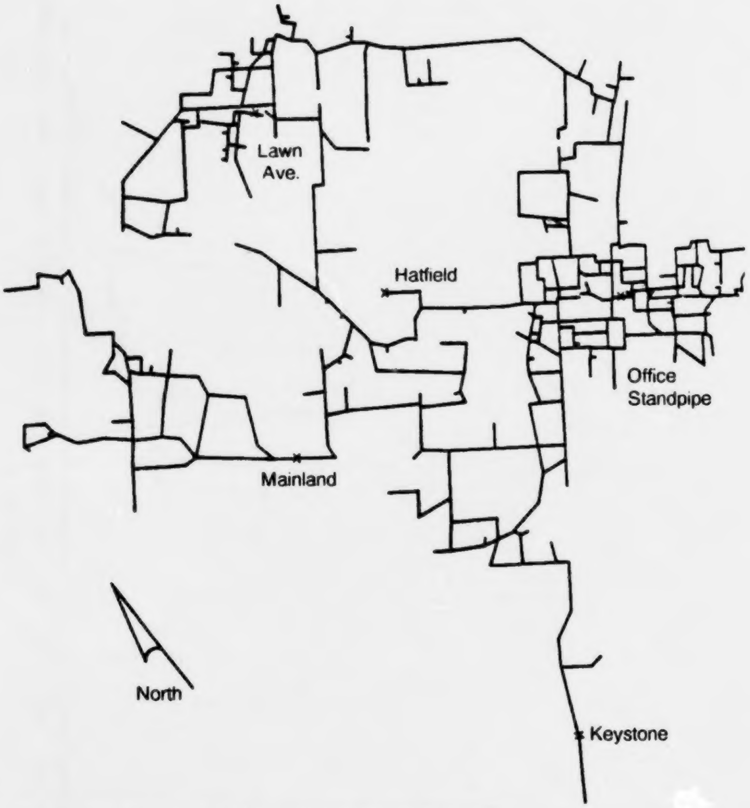


Figure 3.5.22. Sample tracking points in North Penn system.

provided to any point from any source will allow for realistic water quality monitoring strategies.

Future research should include examining the sensitivity of the steady-state models to changes in hydraulic assumptions. Further algorithmic development should be devoted to additional development and testing of dynamic models. This work should also be extended to a study of bacterial contamination from multiple-source and single-source systems with elevated tanks. The model might be helpful to epidemiologists in analyzing exposure profiles for comparison of attack rates.

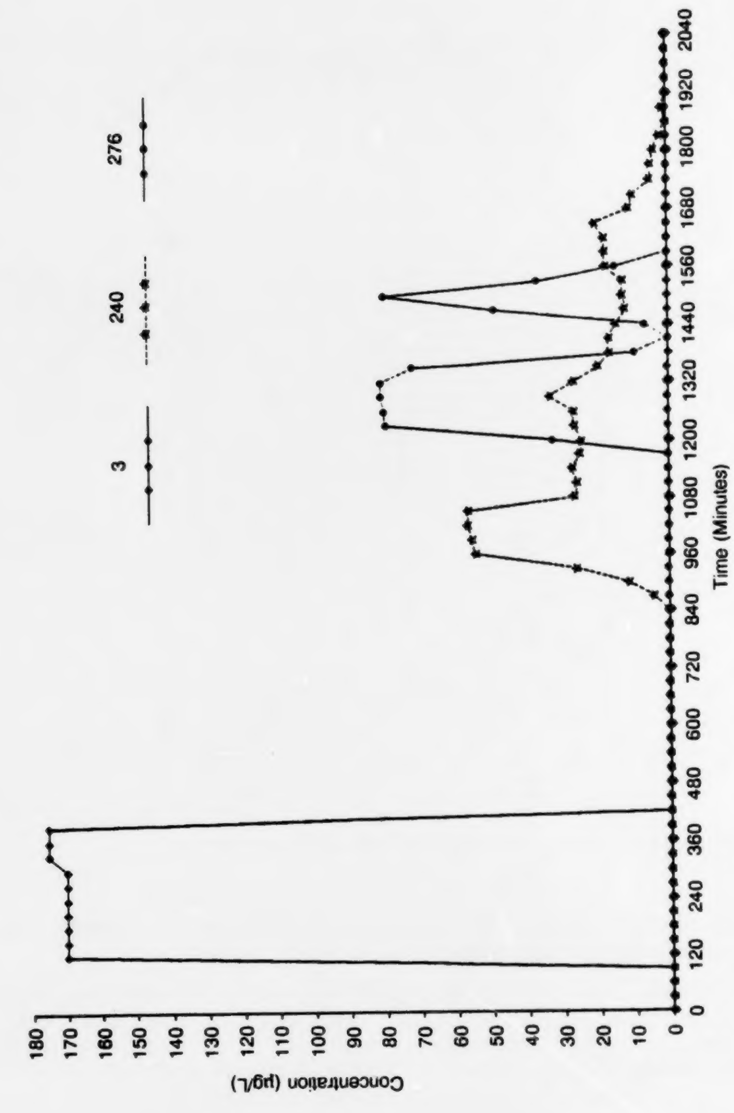


Figure 3.5.23. Prediction of contaminants movement at various locations in North Penn system.

Acknowledgements

The author would like to acknowledge the assistance of Ms. Diane Routledge and Ms. Patricia Pierson in preparing this manuscript.

References

1. Clark, R. M. and Males, R. M. "Simulating cost and quality in water distribution." *J. Water Resour. Plng. and Mgmt.* ASCE, 11(4):454-466, 1985.
2. Chun, D. G. and Selznick, H. L. "Computer modeling of distribution system water quality." *In:* Computer Applications in Water Resources, ASCE, New York, New York, 448-456, June 1985.
3. Gessler, T. and Walski, T. M. Water distribution system optimization. TREL-85-11, Waterways Experiment Station, U.S. Army Corps of Engineers, Vicksburg, Mississippi, October 1985.
4. Clark, R. M. and Males R. M. "Developing and applying the water simulation model." *J. AWWA.* 78(8):61-65, 1986.
5. Males, R. M., Clark, R. M., Wehrman, P. J. and Gates, W. E. "Algorithm for mixing problems in water systems. "*Journal of Hydr. Engrs.*, ASCE, 111(2):206-219, 1985.
6. Males, R. M., Grayman, W. M., and Clark, R. M. "Modeling water quality in distribution systems." *J. Water Resour. Plng. and Mgmt.*, ASCE, 114(2):197-209, 1988.
7. Grayman, W. M., Clark, R. M., and Males, R. M. "Modeling distribution system water quality: A dynamic approach." *J. Water Resour. Plng. and Mgmt.*, ASCE, 114(3):295-312, 1988.
8. Clark, R. M., Grayman, W. M., Males, R. M., and Coyle, J. A. "Modeling contaminant propagation in drinking water distribution systems." *Aqua*, No. 3, 137-151, 1988.

Treatment Plant Evaluation During a Waterborne Outbreak

by: Gary S. Logsdon
Director, Water Process Research
Black & Veatch Engineers-Architects
1025 Alliance Road, Suite 101
Cincinnati, Ohio 45242
(513) 984-6630

[At the time this article was written, Dr. Logsdon was with
the Risk Reduction Engineering Laboratory
U.S. Environmental Protection Agency]

Introduction

Evaluation of a water treatment plant during a waterborne disease outbreak represents a challenge different from many other types of engineering work. During an outbreak a great sense of urgency, or even crisis, can be expected. Disease outbreaks, if caused by a pathogen in drinking water, are likely to involve public notification and recommendations to boil water. News organizations naturally have an interest in the outbreak, its possible causes, and the remedies needed to solve the present problem and prevent future problems. Into this environment comes the engineer selected to evaluate the facility and present recommendations.

This paper has been prepared to serve as a guide to engineers who are called upon to work in the difficult circumstances that are present during a waterborne disease outbreak. When events are moving at a hectic pace, referring to a document prepared when the author had time to reflect on the nature of the work to be done during an evaluation may be helpful to the engineer who is attempting to quickly resolve complex issues on-site. A major portion of this paper has been adapted from "Evaluating Treatment Plants for Particulate Contaminant Removal."(1)

Getting started

The first task for the engineer is to get acquainted. This involves meeting regulatory and public health officials, local government officials, and water utility personnel at both the operating and the

Preceding page blank

management levels. Finally, the engineer must acquaint himself or herself with the treatment facilities. One useful approach to the latter task is to tour the entire plant first to obtain an overview of the situation, and then to go through it again for a detailed inspection. During the initial phase of the investigation the engineer must convey a spirit of cooperation and helpfulness. An outsider is dependent on the good will and assistance of the local persons who are experiencing difficulties, and who may be quite upset because of what is happening. Tact, humility, genuine concern, and a sincere desire to help should go far to gain acceptance for the engineer and support for the evaluation job that needs to be done.

During an outbreak, the water utility staff have to produce water and conduct business as usual, in addition to trying to provide information to numerous regulatory officials, news persons, and the general public. Additional information is also necessary for a successful plant evaluation. To reduce the load on the staff, the engineer can also use other sources of information. The water utility's offices and laboratory often contain much valuable information that would aid the engineer in making a plant evaluation. Facility blueprints and diagrams can be used for preliminary hydraulic evaluations. Water quality records should be obtained. Chemical, microbiological, and physical quality of treated water should be reviewed and compared with corresponding data for raw water. How much improvement in water quality is attained by treatment? How often does raw water vary and what are the influences of raw water quality changes on treated water quality? Factors such as low temperatures in winter, spring runoff, summer and fall algal blooms, and low flow conditions may influence finished water quality. The unique aspects of each water source should be kept in mind as plant evaluations are made.

Review of operating records also can be a useful way to gain an understanding of the water utility's disinfection practice. Information should be available on type of disinfectant used and history of disinfectant doses and residuals. When combined with flow data and information on disinfectant contact time, the residual data can be used to estimate C·T values.

Records of disinfectant dosage and residual should be reviewed. (Ozone is very reactive and residuals for this disinfectant remain in drinking water for only a short time, so most of the following remarks do not apply to ozone.) The quality of disinfectant added per day can be estimated by multiplying the rate of flow by disinfectant concentration at a point shortly downstream of its addition, but after good mixing. This approximation can then be compared to the plant's records for the amount of chemical fed per day. Disinfectant demand can influence this comparison of dose vs. residual, but comparing measured chemical usage vs. calculated usage should reveal gross problems in disinfectant chemical feed control or in the measurement of disinfectant concentrations in water.

Records of residual measurement should be reviewed to determine the frequency with which such measurements are made. If a continuous analyzer is not used, how frequently are grab sample measurements made? The adequacy of this frequency should be considered, and it is somewhat related to the variability in raw water quality. Disinfectant residual should be measured frequently enough to ensure that a change in disinfectant demand has not produced an inadequate residual.

Records should also be studied for indications that disinfectant demand is determined. Sudden changes in disinfectant demand can cause temporary depletion of the residual, and this might allow penetration of the treatment barrier by pathogens.

Reviewing and developing data related to the water utility will consume extra time at the beginning of a treatment plant investigation, but the time invested in this effort can be expected to yield a more productive and insightful evaluation.

Monitoring and Quality Control

Analytical methods and quality assurance deserve careful attention. The methods used should be in accordance with regulatory requirements. Measurements that are very important for clarification plants include coagulant dose, residual coagulant, pH, and turbidity. Disinfectant residual is a key measurement at all plants. A recently published paper gives additional information on the measurement of disinfection residuals (2). Quality assurance procedures should be reviewed, and if necessary, the advice of a chemist familiar with disinfection chemistry should be sought. This is especially true for chlorine dioxide and ozone, both of which can present serious analytical difficulties. The disinfection process is really not under the control of treatment plant staff if they are not able to measure concentrations of disinfectant in the water accurately.

Handling and analysis are important for turbidity samples. These samples should be taken to the laboratory promptly when grab samples are analyzed. The sample cell must be scrupulously clean when it is inserted into the turbidimeter, and there should be no air bubbles in the sample. This can be a problem when the water is cold but it is vital to obtain accurate measurements. Turbidimeters should be calibrated with secondary standards as often as instructions from instrument manufacturers and experience indicate (whichever is shorter). Calibration with formazin or some other approved primary standard should be done as frequently as required by *Standard Methods* (3) or the EPA Methods Manual. Most improper practices in handling low turbidity water samples cause the turbidity measurement to be higher than the actual value. Careful handling and measurement of grab samples may reveal that filtered water turbidity is actually lower than it was thought to be when sufficient analytical precautions were not taken.

When a filtration plant is inspected, the engineer should pay careful attention to the sampling points for filtered water turbidity, to sample handling, and to instrument calibration. To really be able to control filter operation, the plant operator should be able to sample each filter effluent independently, at a point where there has been no mixing with water from any other filter. Although sampling at a point of entry into the distribution system (e.g. clearwell effluent) is required by the turbidity regulation, clearwell sampling reveals very little about the specific condition of an individual filter, because of the blending that occurs in the clearwell.

A review of plant records should show that adequate documentation exists, both for routine monitoring and for the measurements needed for quality control testing. Unless this is available, the validity of the process monitoring data is questionable.

Plant Hydraulics and Flow Patterns

Hudson (4) stated that one of the two categories of deficiencies causing many water treatment plants to fail to perform as well as expected was hydraulic inadequacies, including flow distribution, incorrect baffling, and a lack of consideration for mixing intensity criteria. Because of its great importance, plant hydraulics should not be overlooked.

The quality of filtered water can be affected by plant hydraulics. The adverse influences of changes in rates of flow and flow patterns on water quality can be very important. As flow rate is increased, detention time decreases. Under some circumstances, shorter times for flocculation or sedimentation can lead to poorer water quality. Increased flow rates that result in higher filtration rates may have this effect also, especially if the floc is weak.

Flow metering and measurement are important because evaluating and controlling plant performance are difficult if flows are unknown. In order for a plant to be operated properly the total flow rate has to be known, on an instantaneous basis or by calculations based on volumetric measurement. Is such equipment in place and working? In addition, whenever flow is divided in a plant, some means should be available for operators to understand how the flow is being split.

Flocculators, settling basins, or filters operated in parallel should have provisions for determining flow. Unequal division of flow to two identical settling basins could result in overloading one basin, with poorly settled water as a result.

Pressure filters operated in parallel can be operated in a variable, declining rate mode, with the most clogged filter operating at the slowest filtration rate; however, flow meters should be provided on each filter so operators can determine the actual rate of filtration. Again, if one or more filters is clogged and not carrying a fair share of the load, other filters may be overloaded and operating at excessively high rates. This can lead to turbidity breakthrough.

Even if improper division of flow among basins or filters is not a problem, poor distribution of flow within a basin might be. Flocculation basins should be operated in a plug flow mode so similar mixing energy and time (GT) can be provided to all of the water. An unbaffled flocculating basin resembles a continuously stirred reactor. A properly baffled flocculation basin can have flow characteristics that approach plug flow. Hudson (4), calculated that in a single compartment, continuously stirred basin, 39 percent of the water will pass through the basin in a time shorter than 50 percent of the nominal residence time. In a series of five baffled, continuously stirred basins, only 11 percent of the water will pass through the basin in a time shorter than 50 percent of the nominal residence time.

Because of the importance of hydraulic patterns, short circuiting, and detention times, it may be appropriate to evaluate flows by performing tracer studies. Lippy (5) recommends this approach and provides suggested procedures. Although dyes are very sensitive tracers, their use in drinking water facilities may be inadvisable. Other tracers are available, including lithium, chlorine, and fluoride. Some caution should be applied if fluoride is used as a tracer upstream of the filters. Standard Methods indicates that polyvalent aluminum can complex with fluoride, the extent depending on pH and the relative levels of fluoride and complexing species. In an extensive study of water filtration at Duluth (6), pilot plant studies indicated the addition of fluoride before filtration required an additional 2 to 4 mg/L of alum to obtain the same level of turbidity that could be attained in the filtered water when no fluoride was added. Chlorine can also be used as a tracer, but chlorine demand and the effects of sunlight can influence results. If chlorine demand is satisfied, sunlight is not a factor in closed basins or during hours of darkness.

An excellent guide to evaluation of flow characteristics and residence times is found in "Residence Times in Pretreatment", Chapter 5 of *Water Clarification Processes: Practical Design and Evaluation* (4). Hudson (4) recommended use of step doses, i.e. a sudden increase of tracer fed on a continuous basis, rather than use of a slug dose. The tracer concentration range that must be measured when a step dose is used is much smaller than when a slug dose is used. This usually makes tracer analysis much easier.

Even if hydraulics are satisfactory, problems related to flow can occur. At water filtration plants, capacities for production and storage of finished water should be in balance with demand. That is, the volume of storage should be adequate to equalize or moderate differences between demand and production.

Process equipment that incorporates mixing, flocculation and clarification should be operated with a minimum of rate changes. An example of this type of equipment is the upflow clarifier, which establishes an equilibrium when the settling velocity of floc particles in the sludge blanket equals the rise rate of the water in the basin. Increasing water production in this type of clarifier causes higher upward velocity of the water, and can result in washing out some of the floc in the sludge blanket. That floc then goes on to the filter, where it could cause shorter filter runs or perhaps pass through into the finished water.

Water filters should be run at constant or gradually declining rates, if possible. If storage is inadequate to provide for increases in demand, then frequent rate changes may be imposed on filters. Some changes will of course be necessary from time to time, but if the volume of storage is small enough to require making several filtration rate changes each date, the benefits of increasing this storage to provide smoother plant operation ought to be seriously considered.

Chemical Feed Selection and Control

Deep bed, granular media filters that are operated at rates of 2 gallons per minute per square foot, or greater, will not work efficiently if the raw water is not coagulated properly before it is applied to the filters (7). In many cases, flocculation and sedimentation are also necessary. This has to be understood clearly by the engineer who evaluates the plant and by the operators who are responsible for controlling coagulation and filtration. Shutting off coagulant chemicals when raw water turbidity is lower than the 1 Nephelometric Turbidity Unit (NTU) Maximum Contaminant Level is never a correct opening procedure, although this mistake has been made numerous times in the past.

Adding coagulant chemicals is essential, and maintaining proper control of the coagulation reaction is very important. The efficacy of inorganic coagulants, especially alum, depends on the pH of the water. Chemical dose is important, both for inorganic coagulants and for polymers. Inadequate doses result in inadequate particle destabilization and coagulation. Overdoses of cationic polymers can result in restabilization of particles. Application of the correct dose of coagulant, on the other hand, results in destabilization of particles, leading to effective flocculation, sedimentation, and filtration. A more detailed explanation of these interactions is given in Chapter 6, "Coagulation" in *Coagulation and Filtration: Back to the Basics* (8). Review of this material is recommended.

Close attention should be given to the procedures used to select coagulant dose and pH at a filtration plant. Some utilities base coagulation chemistry on past practice. This may be helpful if careful records are kept and charts are prepared so changes in water chemistry can be identified. Even so, actual dose selection should be done by jar testing or other methods. The initial advice given on coagulation when the plant was started shouldn't be assumed to be valid to perpetuity.

Probably the most common approach to coagulant evaluation is jar testing. For turbid waters, jar test results can be based on the turbidity of the settled water. For low turbidity waters, perhaps 5 to 10 NTU or lower, changes in raw water turbidity may not be sufficient to clearly indicate optimum chemical doses. Also, at direct filtration plants, sedimentation is not practiced, so evaluating settled water is inappropriate for dose selection. Wagner and Hudson (9) suggest the water from jar tests can be filtered through Whatman No. 40 paper after coagulation and flocculation. They report similar results for filtered water turbidity using this procedure, for water from pilot filters, and for water from full scale filters. Neuman (10) emphasized the importance of performing jar tests with test water at the same temperature as water in the plant. This is especially important when cold water is being treated in the plant, because proper pH and doses may be different when this water is warm. Griffith and Williams (11) evaluated jar testing at Phoenix, Arizona, and reported that the mode of addition of coagulant chemical influenced jar test results. Moffett (12) reported higher doses of alum were needed to destabilize colloidal suspensions in jar tests when coagulant was added on top of the water rather than at the impeller. If a chemical is added by mixing into the water, rather than by pouring it on top of the water in the full scale plant, this practice should be mimicked in jar testing. This can be done by using funnels. The dose of coagulant chemical is added and then followed by a rinse of test water in sufficient quantity to displace all coagulant from the stem of the funnel. If rapid mix times are being evaluated, or if the mixing time is short, chemicals should be added to all jars simultaneously. During a plant evaluation, it is a good idea to watch the plant staff perform a jar test. Also inquire about the frequency of jar testing and the bases for deciding when to test, i.e. seasonal changes, change in river flow, changes in turbidity, etc. This information will provide the background needed to suggest improvements to their testing program. Hudson and Wagner (13) have published an excellent paper on performing jar tests and interpreting the results.

Another approach to dose control is based on measurements of electrophoretic mobility. This is based on the magnitude of the electrical charge on the surface of the colloids. If this charge is large, it can cause the particles to repel each other and remain in suspension. If it is small (near zero), particle collisions can occur and these particles will stick together and grow into flocs that settle out, or stick on the filter media.

Electrophoretic mobility can be determined by instruments that measure zeta potential or streaming current potential. Zeta potential measurements are based on the velocity of individual colloids or groups of colloids. Zeta potential is usually determined on grab samples. Streaming current potential is based on the average of the electrophoretic mobility of all of the particles in the water sample. Streaming current detectors analyze discrete samples, but can be set up to do so repeatedly so they approximate continuous, on- line analysis. The use of streaming current detectors to control coagulant dose was evaluated by Dentel and Kingery (14). When electrophoretic mobility techniques are used to adjust coagulant dose, the validity of these approaches should be verified form time to time by performing a series of jar tests or pilot filter studies with doses at, above, and below the recommended concentration.

Some water utilities use pilot filters to determine appropriate coagulant doses. Proprietary equipment used to filter coagulated water after the rapid mixing step was described by Conley and Evers (15). In these systems, turbidity is monitored continuously, and water is filtered at a high rate. When the turbidity of the water produced by the pilot filter is unsatisfactory, a change in coagulation chemistry is needed. Research on a pilot filter concept for dose selection was conducted by Kreissl et al. (16) about two decades ago. Kreissl and his associates operated clean pilot filters at high rates, to simulate conditions that would be encountered when a clogged filter was operated at a normal rate. They were able to determine coagulant dose with the pilot filters, and in addition, were able to obtain information on floc strength and the ability of the system to resist turbidity breakthrough at the end of a filter run. They reported that pilot filter systems could be used to select optimum chemical dosage at filtration plants that did not employ flocculation and sedimentation.

Tests employing pilot filters rather than the electrophoretic mobility measurements, or jar tests, are appropriate for determining floc strength and the proper dose for nonionic polymers. Visible changes in floc formation sometimes can be seen in jar tests, but no data on floc strength or head loss in filters can be obtained this way. Nonionic polymers are often used to bridge smaller floc particles together and form large floc that settles better, or to produce a tougher floc that resists turbidity breakthrough during rate changes. Better control of turbidity and Giardia cyst removal, even during filtration rate increases, was attained (17) in direct filtration research, when alum and 0.1 mg/L of nonionic polymer were used to condition the water. In a later study Logsdon et al. (18) reported that a slightly anionic high molecular weight polymer could strengthen alum floc and assist retention of the floc and cysts in an 0.9 mm effective size anthracite filter. In this case, polymer-conditioned alum floc filtered in

0.9 mm media produced an effluent turbidity (0.10-0.16 NTU) similar to turbidity produced by 0.42 mm sand filtering alum floc (0.08-0.16 NTU) in an earlier experiment. In both cases, filters were ripened and not exhibiting turbidity breakthrough. Use of polymer can sometimes permit production of high quality water in spite of less than optimum facilities or operation.

After appropriate doses of chemicals are selected for coagulation, it logically follows that the capability to feed the chemicals must exist. Techniques for adding chemicals to raw water vary greatly, from those that give poor results to those that provide efficient and effective utilization of these materials. Several aspects must be considered. These include flexibility in the locations and order for adding chemicals, the actual means of adding and dispersing chemicals, techniques for measuring feed rates and thus verifying the doses, and the kinds of chemicals that are fed.

Flexibility should include the capability to add some chemicals at more than one point in the treatment train. Points of addition may include near the beginning of a raw water transmission main, just before a rapid mixer, in a rapid mix tank, just before flocculation, before filtration, after filtration as water flows to a clearwell, and into backwash water. A sufficient number of feed points should be available so that each chemical has the opportunity to mix completely with the water and function as intended rather than react in a concentrated form with some other chemical being added to the water. Hudson (4) suggested the use of plastic water meters or plastic volumetric cylinders to measure actual feed rates on a short term basis.

The actual mode of chemical addition is extremely important. Inorganic coagulants and polymers should be diluted sufficiently to readily dissolve and disperse in the raw water, but they shouldn't be diluted so excessively that they become less effective. This aspect of alum feeding was discussed by Griffith and Williams (11) who reported dilution of liquid alum to a 1.5 percent solution gave them satisfactory results. They also indicated that another investigator found alum diluted to 0.3 percent was less effective. Manufacturers' recommendations should be followed for polymer dilution.

Chemical pumping equipment and piping should be evaluated. A commonly used feed pump is the diaphragm pump, which pumps in pulses. If these pumps are used to add coagulant to raw water flowing in a pipe, and they operate at 60 cycles per minute, with pumping occurring only 0.5 second of each 1.0 second cycle, only half of the water comes in initial contact with alum solution. If water in the pipe is flowing at 6 feet per second, every other 3 foot segment has no alum, while the other segments have twice the desired dose. Alum hydrolyzes in a fraction of a second (19). Thus, some of the chemical reactions will take place before the overdosed and underdosed water are mixed. The pulsed flow pattern from a diaphragm pump can be converted to continuous flow by installation of a vertical pipe which is filled with air and capped or by use of a pneumatic tank (an accumulator) downstream from the pump. These devices should be used with diaphragm pumps to obtain maximum efficiency with coagulant chemicals.

Because of the rapidity with which coagulation occurs, the stream of coagulant chemical must be dispersed throughout the entire quantity of raw water in a nearly instantaneous fashion. Chemicals added in a rapid mix chamber should be delivered close to the impeller. Chemicals fed into a pipe should be fed with jets pointed upstream against the flow. Multiple injection points across the area of flow, as suggested by Forbes et al. (20), are preferred to a single injection point. Another technique for adding chemicals in pipes is the use of orifice plates to create turbulence. Commercially fabricated motionless mixers (static mixers) are also available for in-line rapid mixing. The least preferred way of adding coagulant chemicals is to simply dribble or drop a steam of coagulant onto the surface of the raw water. This is sometimes done in older water plants that employ a rising well for rapid mixing, but improved results can often be obtained by providing more rapid ways of mixing.

The plant evaluator should note the variety of chemicals being fed. These can include inorganic coagulants, polymers, acid, lime, caustic soda, soda ash, corrosion inhibitor, powdered activated carbon, disinfectant, ammonia, and fluoride. The order of chemical addition can be important. For guidance on this, reference works should be checked, or jar tests should be performed. Certain chemicals interfere with others. For example, powdered activated carbon added for taste and odor control may decrease the chlorine residual, polyphosphate added to sequester iron may reduce the efficacy of zinc as a corrosion inhibitor for asbestos cement pipe, (21) and fluoride added before filtration may complex with aluminum and necessitate use of more alum to coagulate the water (6). The sequence used for chemical addition at a specific plant is often based on past practice which has been developed over the years on an ad hoc basis. Conducting jar tests to verify whether this sequence actually gives the best results is advisable.

At diatomaceous earth (DE) filtration plants, coagulant chemicals generally are not used. During the DE filtration process, a small amount of diatomaceous earth (the body feed) is added to the raw water. The DE particles, and the particles in the raw water, are filtered out on the surface of a cake of diatomaceous earth that covers the filter leaves, or septa. Adding DE body feed to the raw water helps to maintain the high porosity of this cake, and thus maintains the good performance characteristics of the filter.

The dose required for body feed is dependent upon the concentration and nature of the particles in the raw water. As raw water particulate matter increases in concentration, the body feed dose is increased to maintain a similar ratio of particles in the filter cake. Rigid particles have good filtration characteristics, whereas gelatinous or compressible particles, such as alum floc or algae, tend to clog the filter cake. The body feed dose for rigid solids may be as low a 1 mg/L of DE per 1 mg/L of solids. For compressible solids, the ratio of DE to solids can be as high as 10 mg/L of DE per 1 mg/L of solids (22).

The grade of DE (size distribution of diatomite particles) is also important. Removal of particles generally occurs by straining, and a smaller pore structure is needed for removal of bacteria than for Giardia cysts. Finer grades of DE are needed for removal of smaller sizes of particles, but the smaller pores result in a more rapid build-up of head-loss. A trade-off exists between better effluent clarity produced by finer grades of DE, and longer filter runs produced by coarser grades.

Rapid Mixing

Moffett (12) called rapid mixing the most important step in the water treatment process. Letterman et al. (23) evaluated rapid mixing and showed that a short period of high intensity mixing was the method of choice. Vrale and Jorden (24) evaluated rapid mixer configurations and for alum coagulation, concluded that in-line mixers (plug flow reactors) were superior, whereas backmix reactors were less effective. Backmixing systems were better for lime softening, because the wide distribution of residence times in these mixers provides some crystallized $CaCO_3$ particles which can promote more rapid precipitation.

Another commonly used approach to rapid mixing is addition of chemicals at a hydraulic jump. If this method is used, the chemicals should be distributed across the entire width of the flow because of the rapidity with which coagulation reactions occur. In addition, these chemicals should be added just upstream of the hydraulic roll because this is the zone of higher velocity and lesser depth.

Many plants may have only one rapid mixer. If sequential additions of chemicals gives superior jar test or pilot plant results, adding more mixing capability may be advisable. Options include in-line mixing in a raw water line before the rapid mix compartment, or perhaps subdividing the original rapid mix compartment. Earlier practice typically provided more residence time in rapid mix basins than is needed for good mixing, so subdividing may be feasible. The amount of energy applied in the mixing basin may influence the quality of the water produced. However, it is not likely that frequent variations in mixing energy will be used by operating personnel. In a plant evaluation, the engineer should consider whether or not large scale changes in mixing energy should be made, rather than try to fine tune the system by factors of 5 to 10 percent.

Flocculation

At filtration plants that employ coagulation, flocculation is the first optional process. In-line direct filtration plants do not employ flocculation. Other direct filtration plants use flocculation but omit sedimentation. Conventional filtration plants use rapid mixing, flocculation, sedimentation, and filtration. At a direct filtration plant, flocculators are operated to produce a small, dense floc that will store well in the filter, whereas at a conventional plant a floc that settles well is desired. These differences in purpose should be considered in a plant evaluation.

Because of the need to have some uniformity in flocculation time, these basins should be well baffled so that plug flow can be approximated. Research by Argaman and Kaufman (25) indicated that for equivalent removal of particulate matter, a four-compartment flocculator required less flocculation time and energy (GT) than a single basin flocculator. They stated, "Systems with equal overall residence times will perform better as the degree of compartmentalization increases." Their work suggests that if performance is poor and a single compartment flocculator is being used, addition of baffles should be undertaken to provide multiple compartments within the same space.

Floc formation and breakup and the basis for tapered energy input are discussed in Chapter 3, "Flocculation" in *Coagulation and Filtration: Back to the Basics* (26). A high energy input at the beginning of flocculation promotes many collisions among the small coagulated particles that have entered the flocculation basin. As they travel through the basin, the floc particles grow. Larger particles need less energy for transport, and excessive energy might even break up the larger flocs. Because flocculation is dependent on both energy input and flocculation time, prevention of short circuiting is important. With completely mixed flow or when short circuiting occurs, a substantial portion of the coagulated water will pass through the flocculation basin in a time less than the theoretical detention time, and thus the GT input for that water will be less than desired. Jar tests can be used to develop preliminary estimates of the flocculation time and energy needed to produce acceptable results in the full scale plant.

The time needed for flocculation at direct filtration plants should be determined on a case by case basis. For example, in order to treat cold water, 30 minutes of flocculation was employed at a direct filtration plant in Springfield, Massachusetts (27). Greater or smaller times may be appropriate in different circumstances.

One indication of the benefits provided by extra detention time when cold water is treated is illustrated by the change in performance observed for a pressure filtration package plant at Cayuga, N.Y. (28).

This plant employed three pressure filtration units. The first tank contained a coarse medium, the second was a multimedia filter, and the third contained granular activated carbon. The first unit was used to promote flocculation and remove the larger suspended solids. The second filter was to reduce turbidity to a fraction of 1 NTU, and the GAC unit was to remove taste-and-odor causing substances. During times of moderate temperature, the units worked as intended, and the first two filters removed 98 to 99 percent of the turbidity- causing particulate matter. Filtered water was less than 0.20 NTU more than 50 percent of the time. During winter, however, when water temperatures were below 5°C, turbidity removal by the coarse media filter and the multi-media filter dropped to about seventy percent. Fortunately the GAC filter did a good job of turbidity removal, and the final filtered water turbidity remained low, but this situation produced a higher solids load on the GAC than was desirable. The reason for this performance may have been inadequate contact time from the point of coagulant addition to the multi-media filtration step.

Cleasby et al. (29) compared direct in-line filtration with coagulation-flocculation-filtration on water obtained from a gravel pit near Ames, Iowa. In parallel experiments, they found that the filter receiving flocculated water had a shorter initial improvement period after backwashing, produced a lower turbidity, and had a lower rate of head loss buildup than the in-line filter. However, the filter receiving flocculated water often developed turbidity breakthrough before the in-line filter did.

Although flocculation may be needed for treatment of cold water, it can be appropriate in other circumstances too. When the Southern Nevada Water System, serving the Las Vegas area, expanded its direct filtration plant from 200 MGD to 400 MGD flocculation was added to reduce alum floc carryover and powdered activated carbon (PAC) breakthrough (30). The original plant was constructed to provide rapid mixing and filtration (31). According to Monscvitz et al. (30), about half of the aluminum added as alum in the flash mixers was being carried over into the finished water during in-line filtration. Also, when PAC was added to control tastes and odors, the filtration rate had to be reduced from 5 to 2 gpm/sf to prevent breakthrough of the PAC. Pilot plant studies showed that 15 to 20 minutes of flocculation would reduce carryover of aluminum and prevent breakthrough of the PAC.

A variety of questions should be considered when flocculation is examined during a plant evaluation. If the plant doesn't employ sedimentation, is flocculation needed? Considering the present state of knowledge about flocculation, and the different results obtained at various locations, this question probably should be resolved by a program of pilot testing. If flocculation is used, is the baffling provided adequate to obtain a narrow distribution of hydraulic detention times? If short circuiting is not a problem, is the energy input sufficient to cause collisions between coagulated particles, but not great enough to break up flocs already formed?

Sedimentation

Sedimentation is a necessary treatment step when the raw water contains large amounts of particulate matter or humic substances (color), or if the coagulation that gives the desired finished water quality results in the production of large volumes of filter clogging floc. If a sedimentation basin is in use, but seems to be ineffective, some questions are in order. One that is not obvious is whether the engineer selected the correct solids separation process in the initial design. When soft, highly colored waters are treated for color and THM precursor removal, a light, fluffy, poorly settling floc is often formed. When algae are present in raw water, floc particles containing algal cells may be floated to the surface of a sedimentation basin by bubbles of oxygen produced during photosynthesis. When floc is resistant to settling, dissolved air flotation may be a more appropriate solids separation step. The evaluator should analyze the reasons for floc behavior and adjust the treatment process to match the situation.

At locations where the floc should settle, but it doesn't, the engineer should look for problems in settling basin design or operation. Settling theory is based upon an idealized concept which assumes the velocity of water in the basin is distributed uniformly, and all of the water progresses form the inlet end to the outlet end of the basin. The minimum settling velocity for particle removal in the basin is calculated by dividing the depth of the water by the transit time for inlet to outlet.

Real world conditions, unfortunately, differ considerably from this ideal situation. Short circuits in settling basins are a fact of life. Among the causes for short circuiting are effects of wind, temperature (and therefore density) differences, inlet design, and outlet design. Finding remedies to poor settling basin behavior can require using a variety of approaches.

Temperature differences and the resulting density currents may be present if large diurnal temperature differences are occurring. Cold nights, sunny days, and a shallow raw water source susceptible to these changing conditions can produce rapid temperature changes in the raw water and in the treatment plant. A series of temperature measurements in the raw water and at various places in the treatment plant, especially across the width and depth of the settling basins, should reveal the extent of these temperature differences and indicate whether this might be a problem.

Checking a settling basin for hydraulics and flow distribution problems can be more involved. Tracer studies may be needed. Another approach is visual observation. Hudson (4) published an aerial photograph showing clouds of floc in a settling basin with a poorly designed inlet. Identification of these localized clouds of floc and close observation of basin outlets will frequently reveal design deficiencies. When viewing underwater objects, greater success generally is attained by looking down from above than by looking across and down into the water. Application of the "look down from above" concept may necessitate viewing settling basins from the tallest nearby structure at the waterworks, or more probably, viewing the basins from a small airplane or helicopter.

After tracer tests and visual observations are made, some physical adjustments may be necessary. If it is possible, some trial and error modifications to baffle arrangements could be attempted before permanent modifications are put in place. If baffle modifications are not sufficient, or if a higher treatment rate is needed, addition of tube settlers should be considered. Conley and Hansen (32) have discussed the addition of tube settlers to existing basins, and have provided guidance for design of these installations.

When settling is evaluated, the engineer should keep in mind Walter Conley's paper, "Integration of the Clarification Process" (7). The goal of treatment is to produce the highest quality filtered water, not the best quality of settled water that can be attained. Thus, improvements made to settling basins, while important, do not constitute the end of the upgrading job. The filters must also be evaluated.

Filtration

The final, and in some plants only, step for removal of particulate matter is filtration. Improperly designed, operated, or maintained filters can contribute to poor water quality, even if pretreatment is good. On the other hand, if pretreatment is wrong, or worse yet, nonexistent, the best rapid rate filter is not going to completely salvage the situation and produce high quality filtered water. Again, the idea of integration of the clarification process is important.

Several aspects of filtration should be investigated. These include filter bed design, filtration rate and the methods used for rate control, filter washing and physical condition of the beds, hydraulics, and water quality monitoring.

Filter bed design has varied through the 1900's. Beds of sand, often with an effective size of about 0.4 or 0.5 mm, were commonly used for about half of this century. Work by a number of researchers (7,33,34), helped to bring multi-media filter beds to the attention of engineers and water utility managers. Dual media (anthracite coal and sand) beds were used to provide larger pore spaces for storage of floc within the depth of the bed, rather than just near the surface, as occurs with rapid sand filters. Even when operated at rates higher than conventional rapid sand filter rates, dual media filters provide excellent filtered water turbidity because of the finer layer of sand below the coal.

Both Conley and Pitman (33) and Robeck et al. (34) reported on the use of polyelectrolyte to toughen the floc and control turbidity breakthrough in the latter phase of the filter run. Conley and Pitman observed polyelectrolytes could be used with sand filters, but the fine sands that produced exceptionally clear water also developed high head loss. Robeck et al. reported that a polyelectrolyte coagulant aid helped achieve the optimum strength of floc, and that adequate floc strength prevented the passage of coliform bacteria, virus, carbon, or floc. Logsdon et al. (18) compared different filter media configurations for turbidity and Giardia cyst removal. They also presented data on the effects of chemical pretreatment on filter run length. Head loss development occurred several times faster in the sand filter, compared to dual media. Estimated times to 8 foot head loss at a 3 gpm/sf filtration rate were 13 and 28 hours with sand filters for runs with alum and high molecular weight, slightly anionic polymer vs. four days with the dual media filters.

If hydraulics for filtration and backwashing permit, conversion of sand filters to dual or tri-mixed media filters may enable a water utility to operate filters at higher rates while maintaining or improving upon effluent turbidity and lengthening filters runs. The feasibility of making such a change should be carefully evaluated by the engineer before modifications are begun. Surface wash should be provided, if it was not used with the original rapid sand filter.

Dual and mixed media concepts were developed so filters would have coarser grains at the top and finer grains at the bottom after backwashing. When a bed of mixed particle sizes of uniform specific gravity is backwashed, the resulting gradation is fine to coarse from top to bottom. Use of appropriately sized filtering materials of different specific gravities ranging from 1.4 for some anthracite media to 4.1 for some garnet media has brought about the desired coarse to fine gradation.

Design of multi-media beds must be approached with caution, especially if conversion of rapid sand filters is contemplated. In this situation, constraints on the backwashing rate may exist. In any case when more than one type of filter media is used, calculations should be made to verify the materials selected have similar fluidization velocities. Cleasby (35) has suggested doing this by calculating the fluidization velocity for the D_{10} size and the D_{90} size (10% by weight smaller than D_{10} and 90% by weight smaller than D_{90}) grains for each filtering material. Fluidization velocities for the D_{10} grain size of all materials should be similar. Fluidization velocities for the larger D_{90} sizes will be greater than the D_{10} velocities, but they too should be similar for all of the filtering materials. A small uniformity coefficient will narrow the range in fluidization velocities for the coarse and fine grains of each material. The circumstance to avoid is having a multimedia filter with one layer that fluidizes at a different backwash rate from that needed by the other layer or layers. If this occurs the plant operator may have to backwash at a rate not adequate to fully clean the layer that is not so readily fluidized, to avoid washing out the easily fluidized layer. Pilot plant studies can be used to demonstrate performance of the design.

Because of the difficulty associated with sampling individual materials in a multi-media filter bed that has been in use, the engineer probably will have to rely on a check of filter media specifications or conduct visual observations during backwashing to look for problems in this area.

Filter rate is an important factor in determining water quality. Rate increases are especially important, because sudden increases can cause deterioration of filtered water quality. Cleasby et al. (36) evaluated the effect of filtration rate changes on effluent quality and reported that both the magnitude of the rate increase and the rapidity of the increase were important. Operators need to understand the concept that gradual increases are less detrimental to quality, and they should manage filtration rate changes accordingly. Logsdon et al. (17) reported than an abrupt rate increase caused higher levels of turbidity and *Giardia* cysts when alum was used, but a stronger floc consisting of alum and nonionic polymer resisted breakthrough during a rate increase. Both of these studies provide strong support for minimizing filtration rate increases and avoiding a stop-start operating mode for filters.

Research on filtration rate increases was conducted at the Duluth Filtration Plant (6). Typical coagulation practice involved use of 10 to 15 mg/L of alum, about 0.10 mg/L of nonionic polymer, and a pH of 6.8 to 7.3, depending on water temperature. Three rate change conditions were evaluated: 1) starting a dirty filter after shutdown overnight by going from 0 to 3.25 or 4.87 gpm/sf in 15 minutes; 2) going for 3.25 to 4.33 gpm/sf or from 4.87 to 6.49 gpm/sf in 60 seconds when one filter was removed from operation for backwashing; and 3) starting a clean filter and going from 0 to 3.25 or 4.87 gpm/sf. Both the dual media filter and the mixed media filter were restarted when clogged (9.0 ft. head loss) and about 2 x 10^6 amphibole fibers per liter (F/L) were detected in the effluent. Slowly restarting dirty filters at lower head loss conditions did not produce this effect. Typical filter effluent during normal operation had amphibole fiber counts of 0.04 x 10^6 F/L or lower.

Filters should be operated continuously from the beginning of a run until terminal head loss is reached, and then washed. Dirty filters should not be restarted (37). Logsdon et al. (17) showed that Giardia cysts previously stored in a filter were discharged during a turbidity breakthrough, when no cysts were present in the influent water. In filtration research in which precipitated iron was the particulate matter to be removed, Cleasby et al. (36) maintained a constant influent iron concentration of 10 mg/L and observed peak effluent concentrations as high as 44 to 135 mg/L under instantaneous rate increases of 25% to 100%. Contaminants stored in a filter can be discharged later if the filter is operated improperly.

When a filtration plant is evaluated, typical plant operations should be observed. Pay close attention to filter start-up, rate changes, and backwashing procedures. Are changes in flow made smoothly and slowly, or rapidly and in jerky steps? The latter will be detrimental to water quality. If a plant has conventional rate of flow controllers, their condition should be checked. Are they well maintained and functioning smoothly, or neglected and erratic, or even unworkable? Hudson (4) noted that under fixed conditions rate controllers may cause surge amplitudes of 2 to 10 percent of head loss. He suggested evaluating this problem by measuring the distance between extreme levels in water piezometer tubes which occur within a one minute period. These measurements should be repeated several times to obtain representative values. Water piezometers are very sensitive indicators of filter surges and they are economical to install. Their use should be considered when filters are studied.

Other aspects of filter hydraulics are also important. Flow measurements should be made for each filter so the operator knows the actual rate of filtration. Has flow measurement capability been provided for each filter? This is not always done. If a flow measurement device exists, does it work, and when was the last time it was calibrated by a reliable method, such as measuring the rate for drawdown of water over the filter? In order for the plant operator to be in control of the filtration process, he or she must know what is going on.

If water quality is seriously degraded at the start of a new filter run at least three remedies may be considered. The simplest approach may be to start a new run very gradually, rather than abruptly. This concept was evaluated by Amirtharajah (38). If the filtration rate is increased uniformly from zero to the desired value over a period of 10 to 30 minutes, the effect of restarting after backwash may be diminished. Another approach, used by Harris (39) at the Contra Costa County Water District was to add nonionic polymer[1] to the backwash water. He indicated turbidity of the initial water produced following backwash was on the order of 0.10 NTU when the filter media was preconditioned with polymer.

Chen (40) studied filter bed conditioning with polymer using the U.S. Environmental Protection Agency's Drinking Water Research Division Pilot Plant. When the facilities were operated in the direct filtration mode, both turbidity and the concentration of particles in the 7 to 12 μm size range could be lowered somewhat at an 8.5 gpm/sf filtration rate and even more at a 5.0 gpm/sf rate, but some quality deterioration occurred at the beginning of the runs in nearly every trial.

---

[1] Magnifloc 985-N. Mention of commercial products does not constitute endorsement.

If these approaches do not work, another way to improve water quality is to practice filter to waste. This may, however, require some extensive physical modifications which could be expensive to implement. Filter to waste may be impractical under some conditions, even if the physical arrangement of the plant permits these modifications. Cleasby et al. (29) reported that when direct in-line filtration was practiced with a cationic polymer as the primary coagulant, the initial turbidity improvement period lasted several hours in some runs. Filter to waste could not be used for such a long time. In cases like this, a better approach will be to change coagulation practice so that the ripening or improvement period is drastically shortened.

During a plant evaluation, the condition of the filter beds should be determined by examining a filter that is about ready to be backwashed. After the water is drawn down below the media surface, the engineer should climb down into the filter for a close look. Is the surface of the bed level, or do obvious hills and valleys exist? If so, this indicates problems with bad flow distribution during backwashing or filter operation. Does the filter surface contain mud balls or caked mud? This indicates cleaning during backwashing may be inadequate.

After the filter has been washed, the filter should be drawn down again so the inspection can be repeated. At this time the cleanliness of the media can be evaluated. Filter media should be clean after backwashing. Clean grains of sand, coal, or garnet will not be noticeably cohesive, even when wet. If filter media can be squeezed by and into a ball, dropped on to the filter bed from waist height, and remain intact; or if filter media is spongy when squeezed into a ball it may not be as clean as it should be to function properly.

When a filter is being backwashed the engineer should observe it carefully, watching for sand boils at the beginning of the wash. Baylis, Gullans, and Hudson (41) wrote that filter backwashing should begin slowly and be increased with care. They felt that at least 30 seconds should be allowed to bring the backwash flow to its full value. Serious support gravel disturbances or disruption of false-bottom filter underdrains can be produced by sudden application of the full flow of backwash water.

Some form of washing assistance is necessary, because backwash alone usually does not adequately clean filter media. U.S. practice generally has been to use surface wash, although air-assisted backwashing has been used successfully in some plants. Performance of surface wash also should be observed. The objective of this inspection is to verify that the water flow from the washing device is uniform and the arms are rotating smoothly. surface wash spray nozzles can become clogged during use and should be inspected routinely. Polymer floc tend to be more sticky than alum floc, so if polymers are used to assist in conditioning the raw water, careful attention to the surface wash is certainly appropriate.

When air scour is used, careful operation is essential. Introducing air into the filter too abruptly can damage the filter bottom. Simultaneous application of both air and wash water has the potential to cause media to wash out of the filter bed. If wash water is applied to carry away dirt removed from the filter by air scour, the washing should be very gentle during the air scour step and for as long as air remains in the bed. Dirt loosened by the scouring process can be carried away at velocities lower than those needed for fluidization of media. Fluidization, if practiced, should occur only after the air has been removed from the filter bed.

A key aspect to attaining the best possible performance of filters is adequate monitoring and control. Filtered water turbidity, at a plant that practices effective coagulation, can give an indication of the efficacy of *Giardia* cyst removal (17,18,42); asbestos fiber removal (43), and of particle removal in general, even though concentrations of cysts or fibers are below the levels that can be detected by a turbidimeter. Research results suggest that when filtered water turbidity is very low, removal of cysts or fibers is effective, as is removal of light scattering particles in general. One differing view of the value of turbidity measurements has been presented by Brazos et al. (44) who contended there was very little relationship between turbidity reduction and removal of total bacterial cells as determined by direct microscopic count.

Hudson (4) stated that much credit for improvement in water quality is due to the development of reliable water quality monitoring devices, including turbidimeters. He wrote:

> "In a number of plants, filtered-water turbidity levels prior to the initiation of turbidity monitoring were commonly held in the range of 0.2-0.5 NTU. After the initiation of monitoring, operators could observe episodes of quality deterioration and develop techniques to prevent such episodes, gradually revising their personal quality goals to new levels and commonly reducing the filtered-water turbidity to 0.02-0.05 NTU, an order-of-magnitude improvement. This process takes one to two years, but once having become accustomed to the production of water quality at such levels, the operators of these plants become intolerant of filtered water with more than about 0.06 NTU."
>
> "One of the axioms of water quality control is that, as the clarity of water is improved by improved treatment, there is a parallel reduction of color, taste and odor, bacteria and viruses,

and often of iron, manganese and alumina levels. While few consumers can detect turbidity at a level of 1 NTU except in bathtubs or swimming pools, the use of much lower levels of turbidity brings about a corollary improvement of other water quality parameters. The turbidity measurement is quick and convenient, and although it is used at levels lower than those of consumer awareness, it provides a most useful, rapid means of control of treatment processes."

A similar sentiment was recently expressed. James R. English (45) wrote in a letter to the Editor AWWA's *Mainstream* that turbidity of filtered water at the Sidney N. Peterson Water Treatment Plant operated by the San Juan Suburban Water District in Roseville, California averaged 0.04 NTU. He also stated, "Achieving the levels of water quality we have has completely changed the self-image of each plant operator. They begin to worry when turbidity levels reach 0.06 NTU, and when that happens, they typically find that a drift has occurred in the chemical feed." This sounds very much like Hudson's comment on operator attitudes. Plant operators should continue to strive to find better, more cost-effective ways to treat water and improve finished water quality.

Other evidence of the attainability of very low filtered water turbidity was presented by Schleppenbach (6). The Duluth Filtration Plant produced water with effluent turbidity consistently below 0.10 NTU for over two years, after the initial start-up and learning period at the new plant. This occurred over all four seasons, and a range of water temperatures from 1°C to about 12°C.

A key to success at Duluth, and an approach used by many filtration plants, is the continuous monitoring of effluent turbidity. Conley (7) stated, "Continuous monitoring of filter plant effluent with sensitive turbidimeters is essential for intelligent management of the plant." A similar opinion was held by Robeck et. al. (34) who wrote, "Of course, monitoring for turbidity passage on a continuous, or half hour interval, basis also allows the operator to know the status of the filters and the influence of chemical dose changes."

"Intelligent management", and "knowing the status" are the key words. When turbidity is monitored consistently, operators are able to observe trends in turbidity, and changes can be acted upon soon after they occur. The operator is not put in the position of knowing what the turbidity is only once every two, four, or eight hours, as might be the case when turbidity is measured only in grab samples taken to the laboratory for analysis. Grab sample measurements may be needed for compliance purposes, and they can be used as a means of adjusting the calibration of continuous flow turbidimeters. However, there are many benefits associated with continuous measurements. When turbidimeters are installed on all filters, readout devices can be placed in operational control panels so the operator has instantly available information to act upon. Chart recorders can be used to provide written records which are useful for compliance purposes and periodic reviews of the performance of the plant.

The diatomaceous earth filtration and slow sand filtration processes differ in numerous ways from the coagulation-filtration process which has been the subject of much of this paper. Coagulation for pretreatment is not employed in either slow sand filtration or DE filtration, and this difference is crucial. Many of the steps that are part of the evaluation of a conventional plant or direct filtration plant employing coagulation can be omitted. These include: coagulation dose determination, coagulant feed, rapid mix, flocculation, and sedimentation. However, in all filtration processes flow patterns, hydraulics, rate changes, and cycles of on- off operation are very important. These factors need to be considered in the evaluation of any kind of filter. Other aspects of filtration are process-specific and relate to slow sand or DE facilities. They are discussed in the paragraphs that follow.

Slow sand filtration relies extensively on the development of a biological population within the filter to accomplish removal of particulate contaminants. Therefore applying disinfectant chemical to raw water ahead of a slow sand filter can be detrimental to filtered water quality. Because slow sand filters frequently treat raw water that has been given no pretreatment, few options are available to the operator of a slow sand filter plant that is not providing water of satisfactory quality. These filters generally are designed to treat water within a certain range of conditions. Those who attempt to use a slow sand filter outside the established conditions are inviting problems.

Three important factors in successful operation are proper media size, adequate media depth, and proper filtration rate. Proper media design for a new plant is the design engineer's responsibility. However, after a sufficient quantity of sand has been scraped away during the cleaning process sand is again added. This process, called resanding, is often done by the utility without the assistance of an engineer. Slow sand filters need media with an effective size (e.s.) of 0.15 to 0.30 mm (46); therefore, resanding with sand intended for a rapid sand filter (0.45 to 0.55 mm e.s.) can cause deterioration of the effluent. An engineer evaluating a slow sand filter should try to learn what size sand is in use.

Scraping a filter repeatedly results in a progressively shallower bed. Failure to resand can have very serious consequences. Visscher et al. (46) suggest that the minimum depth of the sand bed should be 0.5 to 0.6 m. Scraping sand away and failing to replace it periodically will eventually cause the bed to be too shallow to be effective. Finally, in an attempt to obtain higher water production, the utility may operate the filter at a rate which results in reduced efficiency for removal of turbidity and microorganisms. Visscher et al. recommend a range of 0.1 m/hr to 0.2 m/hr for slow sand filters.

If a waterborne disease outbreak has been caused by passage of microorganisms through a slow sand filter, the investigating engineer should base his or her actions on the probability that something related to the condition or operation of the filter is seriously wrong. Historically slow sand filters have provided very good protection against the transmission of waterborne disease. If a slow sand filter is not performing effectively, the filter beds should be dewatered one at a time, and the condition of the sand beds should be evaluated. The sand surface should be level (observe this as the water surface drops below the sand) with no erosion of sand caused by influent water. The sand-wall interface should be free of cracks where water could short circuit the bed and avoid filtration. Core samples could be taken at a variety of locations within the bed to provide data on sand size and depth to the support gravel.

Because slow sand filtration is not widely used in the United States, many water treatment engineers have little or no experience in working with the process. Information on slow sand filtration has been published in JAWWA from time to time since December, 1984, when a number of papers on the topic appeared in a single issue. The International Reference Centre's book on slow sand filtration (46) is also an excellent reference work. *Slow Sand Filtration* by Huisman and Wood (47) published in 1974 is older but very helpful. This is available from the World Health Organization and WHO publication sources. Engineers without slow sand experience should find these sources to be quite useful.

Diatomaceous earth filtration generally does not involve the use of coagulant chemicals to pretreat the raw water. Gelatinous particulate matter tends to blind the DE filter cake, so using alum or iron to treat the raw water would cause shorter filter runs. The size of particulate matter passed by the filter is determined by the grade of DE employed. Fine potable water grades can be used to remove bacteria. Coarse potable water grades will remove *Giardia* cysts. The removal mechanism for cysts is straining (48). For effective virus removal, the diatomaceous earth should be conditioned with a coagulant to promote attachment of virus particles onto the DE.

Passage of microorganisms can be caused by using a grade of DE which is too coarse to remove the organisms in question and by a number of equipment and procedural problems. The filter leaves, or septa, must be kept very clean. Backwash at the end of a run should be thorough. Septa should be inspected visually at periodic intervals. With certain types of filters this can be easily accomplished, but pressure filters with bolted pressure domes on both ends of the cylinder can be very difficult to open and inspect. Filter septum integrity must also be maintained. A hole in a septum can permit raw water to short circuit past the filter cake instead of flowing through it. A precoat thickness of 0.1 Kg/m^2 (0.2 pounds/ft^2) is recommended for *Giardia* cyst removal, and body feed always should be used (17). Any interruption of a DE filter run should be followed by backwashing and precoating the filter. Reuse of dirty diatomite could result in penetration of the DE filter by microorganisms.

Diatomaceous earth filtration, like slow sand filtration, has not been as widely employed as coagulation-filtration. Additional information on DE filtration is available in AWWA Manual M30 (22) and in Chapter 15, "Precoat Filtration," (49) in *Water Treatment Plant Design*.

Disinfection

Disinfection is a key factor in the evaluation of a treatment plant. Among the tasks that need to be accomplished are review of plant records and monitoring procedures (already discussed), evaluation of disinfection equipment, and inspection of physical processes.

The four chemical disinfectants most commonly used in the United States are free chlorine, chloramine, chlorine dioxide and ozone. Although ozone was seldom used in past decades, interest in ozone is rising. A number of new installations have been completed in recent years, and more seem likely to be built. Ozone differs from other chemical disinfectants in its lack of a long lasting residual and in its high level of efficacy. Like chlorine dioxide, ozone must be generated on site.

Review of disinfection equipment is an important aspect of a plant evaluation. If the disinfectant is purchased and fed, the feed facilities should be adequate to provide the needed dose, and a sufficiently large inventory should be on hand. If regulatory requirements are established on the size of this inventory, they should be followed. If the disinfectant is produced on-site, the production capacity of the equipment should be large enough to satisfy the expected disinfectant demand and maintain the desired residual, with allowances for maintenance and repair of the system. On-site production is necessary for ozone and chlorine dioxide, and is sometimes used for chlorine.

The dependability of the equipment is important because disinfectant must function properly any time water is produced. Rugged and reliable disinfection equipment is especially needed at facilities which are remote or are staffed on a part-time basis rather than around the clock. Redundancy of facilities and availability of emergency power should be evaluated also. The type of monitoring equipment and its reliability are important to ensure continuous disinfection with an appropriate concentration of chemical. Because emergencies can occur, disinfection facilities should be provided with alarms and sensors to detect problems of inadequate residual, loss of feed, or other similar situations, such as an inadequate amount of chlorine remaining in a storage tank (50).

Physical processes and facilities play an important role in disinfection. Ideally, disinfectant should be added to water and immediately mixed thoroughly. Following initial mixing, contact time should be provided. The actual contact time is most easily defined and is most uniform when plug flow conditions occur.

Disinfectants should be added at locations where they are compatible with the entire process train and with other chemicals that are being added at the same point. For example, adding powdered activated carbon and chlorine at the same location is a wasteful use of both chlorine and carbon. Adding chlorine to an iron-bearing well water ahead of an aeration tower might be inappropriate if the chlorine oxidized all of the iron and the aerator then served no useful function. Adding chlorine in the plug flow conditions that prevail in the middle of a settling basin would be counter productive, because the chlorine would not mix with all of the water until it flowed out of the basin. Sometimes in water treatment plant design the disinfection process has been "added on" rather than specifically engineered. This can be dangerous if all of the essential concepts have not been considered.

When chlorine is added to water, it should be mixed quickly, thoroughly, and uniformly throughout the water. According to *Water Treatment Plant Design* (51), if the ammonia concentration in water equals or exceeds about 10 percent of the chlorine dose, initial mixing of chlorine is especially important. Failure to attain thorough and rapid dispersal and mixing of chlorine can result in break point chlorination where some portions of the water have substantially higher than average concentration of chlorine and other portions have opportunity for little or no reaction with much lower than average or minimal concentration of chlorine. In the presence of ammonia, chlorine appears to be more effective during the first few seconds of addition, hence the greater importance of mixing that is accomplished very rapidly and with a minimum of backmixing. In-line mixers, diffusers across pipes, and hydraulic jumps (51) would be appropriate, but traditional stirred rapid mix basins with backmixing are not desirable in this circumstance.

At some utilities, clearwells have been used to provide disinfectant contact time. Contact time is variable in clearwells because they are also used as flow equalization basins, storing water when production exceeds demand and providing water when demand exceeds production. Contact times approach a minimum during periods of high flow when the volume of water in storage is low and the clearwell is emptying faster than it is being filled. Even with adequate baffles to attain plug flow, the contact time in a clearwell may not be adequate if this structure is used to provide disinfectant C·T.

C·T Determination

Use of the C·T concept (concentration of disinfectant (C) multiplied by contact time (T) in minutes) is required for unfiltered surface waters in the Surface Water Treatment Rule and is recommended by EPA for plants that filter surface water. Contact time is a function of the flow rate in a treatment plant, contact basin, or pipeline. Accurately estimating this contact time is as important as accurately measuring the disinfectant residual.

Contact time in pipes, basins and tanks is related to flow, volume, and vessel shape. When plug flow is closely approximated, the actual contact time closely approximates the theoretical detention time. According to *Water Treatment Principles and Design* (50) baffled serpentine contact chambers having length to width ratios exceeding 200 give a close approximation of plug flow.

Basins having other configurations, such as clearwells, sedimentation basins, and flocculation basins probably need to be evaluated by tracer test to determine the residence time. Dead spots and short circuiting can occur allowing portions of the water to flow through these basins in considerably shorter times than the theoretical detention time that would be calculated by dividing the basin volume by the flow rate.

Lippy (52) presented an interesting example of theoretical detection time vs. actual contact time in a review of a giardiasis outbreak in New Hampshire. The treatment plant had a 0.5 million gallon storage facility plus a 0.04 million gallon pumping well that followed the postchlorination injection point. Based on volume displacement calculations the 0.54 million gallons should have provided 7 hours of contact time. In actual practice, a substantial boost in chlorine dosage was followed by a measurable increase in the chlorine concentration of the water discharged from the pumping well only 1-3/4 hours later. Contact time is not always what it appears to be. Actual contact times measured by tracer studies should be used to evaluate disinfection practice unless plug flow conditions are know to exist.

After the contact time and disinfectant residual are determined, the C·T. product can be calculated. C·T. values needed for inactivation of microorganisms vary depending on water temperature. The efficacy of free chlorine and chloramine is also dependent upon pH, so both temperature and pH data are needed to judge efficacy of the C·T. product. Records of water quality measurements should be reviewed to determine these parameters.

Summary

Well-operated water filtration plants and properly managed disinfection processes should present effective barriers to the passage of microorganisms into drinking water, especially when both filtration and disinfection are used. Occurrence of a waterborne outbreak is evidence that severe problems have developed, and corrective action is needed. This paper presents a discussion of measures that can be taken by an engineer investigating a treatment plant during or after an outbreak.

References

1. Logsdon, G. S., Evaluating treatment plants for particulate contaminant removal, *J. AWWA*, *79*:9:82- 92, 1987.
2. Gordon, G., Cooper, W. J., Rice, R. G. and Pacey, G. E., Methods of measuring disinfectant residuals, *J. AWWA*, *80*:9:94-108, 1988.
3. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 17th Ed., APHA, Washington, D.C., 1989.
4. Hudson, H. E., Jr. (1981) Water Clarification Processes: Practical Design and Evaluation. Van Nostrand Reinhold Company, New York.
5. Lippy, E. C., Engineering aspects of waterborne outbreak investigation, *In:* G. F. Craun, (ed.), Waterborne Diseases in the United States, CRC Press, Boca Raton, Florida, 1986.
6. Schleppenbach, F. X., Water filtration at Duluth, EPA-600/2-84-083, U. S. Environmental Protection Agency, Cincinnati, Ohio, 1984.
7. Conley, W. R., Jr., Integration of the clarification process, *J. AWWA*, 57:10:1333-1345, 1965.
8. Edzwald, J. K., Coagulation, *In:* Coagulation and Filtration: Back to the Basics, AWWA Seminar Proceedings, No. 20155, 23-44, 1981.
9. Wagner, E. G., and Hudson, H. E., Jr., Low-dosage high-rate direct filtration, *J. AWWA*, *74*:5:256- 261, 1982.
10. Neuman, W. E., Optimizing coagulation with pilot filters and zeta potential, *J. AWWA*, *73*:9:472-475, 1981.
11. Griffith, J. D., and Williams, R. G., Application of jar test analysis at Phoenix, Arizona, *J. AWWA*, *64*:12:825-830, 1972.
12. Moffett, J. W., The chemistry of high-rate water treatment, *J. AWWA*, *60*:11:1255-1270, 1968.
13. Hudson, H. E., Jr., and Wagner, E. G., Conduct and uses of jar testing, *J. AWWA*, *73*:4:218-223, 1981.

14. Dentel, S. and Kingery, K. M., Use of streaming current detectors in water treatment, *J. AWWA*. *81*:3:85-94, 1989.

15. Conley, W. R., and Evers, R. H., Coagulation control, *J. AWWA*, *60*:2:165-174, 1968.

16. Kreissl, J. F., Robeck, G. G., and Sommerville, G. A., Use of pilot filters to predict optimum chemical feeds, *J. AWWA*, 60:3:299-314, 1968.

17. Logsdon, G. S., Symons, J. M., Hoye, R. L., and Arozarena, M. M., Alternative filtration methods for removal of *Giardia* cysts and cyst models, *J. AWWA*, *73*:2:111-118, 1981.

18. Logsdon, G. S., Thurman, V. C., Frindt, E. S., and Stoecker, J. G., Evaluating sedimentation and various filter media for removal of *Giardia* cysts, *J. AWWA*, *77*:2:61-66, 1985.

19. Hudson, H. E., Jr., and Wolfner, J. P., Design of mixing and flocculating basins, *J. AWWA*, *59*:10:1257-1267, 1967.

20. Forbes, R. E., Nickerson, G. L., Hudson, H. E., Jr., and Wagner, E. G., Upgrading water treatment plants: an alternative to new construction, *J. AWWA*, *72*:5:254-261, 1980.

21. Weston Water Utility, Schofield, Wisconsin, Control of Asbestos Fiber Loss from Asbestos-Cement Watermain, EPA-600/2-84-014, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1984.

22. McIndoe, R. W., Logsdon, G. S., Ris, J. L. and Wirsig, A., Precoat Filtration, AWWA Manual M 30, American Water Works Association, Denver, Colorado, 1988.

23. Letterman, R. D., Quon, J. E., and Gemmell, R. S., Influence of rapid mix parameters on flocculation, *J. AWWA*, *65*:11:716-722, 1973.

24. Vrale, L., and Jorden, R. M., Rapid mixing in water treatment, *J. AWWA*, *63*:1:52-58, 1971.

25. Argaman, Y., and Kaufman, W. J., Turbulence and flocculation, *J. of the Sanitary Engineering Division*, ASCE, 96:2:223-241, 1970.

26. Letterman, R. D., Flocculation, *In:* Coagulation and Filtration: Back to the Basics, AWWA Seminar Proceedings, No. 20155, 45-47, 1981.

27. Sweeney, G. E., and Prendiville, P. W., Direct filtration: an economic answer to a city's water needs, *J. AWWA*, *66*:2:65-71, 1974.

28. MacNeill, J. S., Jr., and MacNeill, A., Feasibility Study of Alternative Technology for Small Community Water Supply,

EPA-600/2-84-191, U. S. Environmental Protection Agency, Cincinnati, Ohio, 1985.

29. Cleasby, J. L., Hilmoe, D. J., Dimitracopoulos, C. J., and Diaz-Bossio, L. M., Effective Filtration Methods for Small Water Supplies, EPA 600/2-84-088, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1984.

30. Monscvitz, J. T., Rexing, D. J., Williams, R. G., and Heckler, J., Some practical experience in direct filtration, *J. AWWA*, *70*:10:584-588, 1978.

31. Spink, C. M., and Monscvitz, J. T., Design and operation of a 200-MGD direct filtration facility, *J. AWWA*, *66*:2:127-132, 1974.

32. Conley, W. E., and Hansen, S. P., Advanced Techniques for Suspended Solids Removal, *In:* R. L. Sanks, (ed.), Water Treatment Plant Design for the Practicing Engineer, Ann Arbor Science Publishers, Ann Arbor, Michigan, 1978.

33. Conley, W. R., and Pitman, R. W., Test program for filter evaluation at Hanford, *J. AWWA*, *52*:2:205- 214, 1960.

34. Robeck, G. G., Dostal, K. A., and Woodward, R. L., Studies of modifications in water filtration, *J. AWWA*, *56*:2:198-213, 1964.

35. Cleasby, J. L., Unconventional Filtration Rates, Media, and Backwashing Techniques, *In:* E. A. Glysson, D. E. Swan, and E. J. Way, (eds.), Innovations in the Water and Wastewater Fields, Butterworth Publishers, Boston, Massachusetts, 1985.

36. Cleasby, J. L., Williamson, M. M., and Baumann, E. R., Effect of filtration rate changes on quality, *J. AWWA*, *55*:7:869-880, 1963.

37. Logsdon, G. S., Mason, L. and Stanley, J. B., Jr., Troubleshooting an existing treatment plant, *In:* Filtration: Meeting New Standards, AWWA Seminar Proceedings No. 20028, 109-125, 1988.

38. Bucklin, K., Amirtharajah, A., and Cranston, K. O., The Characteristics of Initial Effluent Quality and Its Implications for the Filter to Waste Procedure, AWWA Research Foundation Research Report, AWWARF, Denver, Colorado, 1988.

39. Harris, W. L., High-rate filter efficiency, *J. AWWA*, *62*:8:515-519, 1970.

40. Chen, Cheng-Tyng, Filter Preconditioning to Reduce Initial Degradation in Effluent Water Quality, Master of Science Thesis submitted to University of Cincinnati, Cincinnati, Ohio, 1986.

41. Baylis, J. R., Gullans, O., and Hudson, H. E., Jr., Filtration, *In:* Water Quality and Treatment, Third Edition, American Water Works Association, 1971.

42. Al-Ani, M. Y., Hendricks, D. W., Logsdon, G. S., and Hibler, C. P., Removing *Giardia* cysts from low turbidity waters by rapid rate filtration, *J. AWWA*, *78*:5:66-73, 1986.

43. Logsdon, G. S., Symons, J. M., and Sorg, T. J., Monitoring water filters for asbestos removal, *J. of the Environmental Engineering Division*, ASCE, *107*:6:1297-1315, 1981.

44. Brazos, B. J., O'Connor, J. T., and Lenau, C. W., Seasonal Effect of Total Bacterial Removals in a Rapid Sand Filtration Plant, *In:* Proceedings 1986 AWWA Water Quality Technology Conference, 795-833, 1986.

45. English, J. R., Meeting turbidity standards, Letter to Editor of AWWA Mainstream, *31*:1:2, 1987.

46. Visscher, J. T., Paramasivam, R., Raman, A., and Heijnen, H. A., Slow Sand Filtration for Community Water Supply, Technical Paper 24, International Reference Centre for Community Water Supply and Sanitation, The Hague, The Netherlands, 1987.

47. Huisman, L. and Wood, W.E., Slow Sand Filtration, World Health Organization, Geneva Switzerland, 1974.

48. Walton, H.G., Diatomite filtration: why it removes Giardia from water, *In:* P.M. Wallis and B.R. Hammond, (eds.), Advances in Giardia Research, The University of Calgary Press, Calgary, Alberta, 113-116, 1988.

49. Baumann, E. R., Precoat Filtration, *In:* R. L. Sanks, (ed.), Water Treatment Plant Design, Ann Arbor Science Publishers, Inc., 313-370, 1978.

50. James M. Montgomery Consulting Engineers, Inc., Water Treatment Principles & Design, John Wiley & Sons, New York, N.Y., 1985.

51. American Society of Civil Engineers and American Water Works Association, Water Treatment Plant Design, 2nd Ed., McGraw-Hill, New York, N.Y., 1990.

52. Lippy, E. C., Tracing a giardiasis outbreak at Berlin, New Hampshire, *J. AWWA*, *70*:9:512-520, 1978. Magnifloc 985-N. Mention of commercial products does not constitute endorsement.

IV. Engineering and Water Quality Concerns

Surface Water Source Protection

by: Joseph L. Glicker, P.E.
Water Quality Director
Portland, Oregon Water Bureau
1120 SW 5th Avenue
Portland, OR 97204
(503) 796-7471

Maintaining the best possible water quality is the goal of any surface water source protection program. This chapter outlines the process by which a surface water source protection program can be developed, the most common threats to water quality in surface supplies, and the general means used to control those threats. It also describes some of the upcoming problems and needs in the field of watershed protection. The City of Portland, Oregon's, Bull Run watershed is used to illustrate application of some of these concepts.

Why Watershed Protection?

Providing high quality water at the consumer's tap is both a desire and a legal requirement for water suppliers in the United States. In meeting this need and desire, a water utility can rely on only two things - the initial quality of its raw water and the treatment and control processes that are applied to that water as the water moves towards the tap.

While water treatment processes can significantly reduce the amount of contaminants present in a raw water, the costs and the potential risks from residual amounts of contaminants left after treatment may be too high. This is particularly true as the number of contaminants of concern increases and the level of risk that the public is willing to accept decreases. Some water systems may require additional steps in the treatment train, such as aeration or granular activated carbon (GAG) filtration, at great increases in treatment costs. The efficacy of these treatment processes is usually measured by the percentage of contamination in the raw water they are capable of removing and not necessarily by the absolute contaminant level they attain. Thus, even with the addition of these treatment steps, the risks of chronic or acute disease from the contaminants remaining in the raw water may still be greater than the consumer is willing to accept. Allowing degradation of a water source because of the presence of, or the ability to construct, a water treatment plant, therefore, will not maximize protection of public health.

A watershed protection program is an important element of the multiple barrier approach to providing high quality water. Watershed protection programs minimize the need for more extensive treatment processes and therefore, minimize the resulting public health risks associated with the existing barriers. Such programs are useful to all water systems regardless of the status or nature of their treatment processes.

Developing a Watershed Protection Program

The first step in developing an effective watershed protection program is to be thoroughly familiar with the watershed and the problems that it faces. The water supplier should determine watershed boundaries, physical features, land uses, hydrology, geology, and their relationship to the water supply facilities. Most importantly, the sources of contamination should be identified and their impact on water quality within the watershed studied. Formal sanitary surveys are a useful tool in this process.

Before a watershed protection program can be developed, the basic goals, principles, and use patterns for the watershed must be identified. These goals will greatly impact the choice of control strategies and protection measures. Examples of types of goals for different watershed protection programs include:

1. Preventing any human-caused situations, such as a hazardous material spill or an aerial pesticide spray program, from contaminating the supply.
2. Mitigating the effects of any natural disasters (flood, fire, windstorms) that may occur.
3. Providing a specific water quality to minimize treatment processes (for example, to be able to use the water with only disinfection or with only conventional treatment but without GAG).

Once the aims of the watershed protection program have been identified, the watershed has been characterized, and the sources of contamination inventoried and studied, the water utility can begin developing specific means of achieving the goals. These means may include physical facilities such as stormwater sediment traps or containment facilities around hazardous material storage sites; operational programs such as inspecting septic tank designs; and management controls such as lot size restrictions or buffer setbacks from streams and reservoirs.

The final step in developing a watershed protection program is monitoring and evaluating the chosen control measures to determine if the identified objectives are being achieved. Any deficiencies noted by the monitoring and evaluation process should then be corrected and improved through ongoing upgrading of the watershed control program.

Land Use Impacts on Water Quality

When using a process like the one outlined above, the water utility will likely find that some of the water quality and land use concerns found across the country will apply. A national survey of surface water utilities conducted in the early 1980s (1) found that agricultural cropland was the primary rural source of water quality problems, followed by forestry activities, feedlots, and large dairy operations and mining. The most significant urban sources of contamination were stormwater runoff from urban and industrial areas and septic tanks. Other sources of contamination include grazing; recreation; municipal and industrial point source discharges; commercial and residential establishments and communities; and secondary uses of water supply lakes, reservoirs, and intake streams for flood control, hydropower generation, recreation, wildlife habitat, and irrigation.

These activities can impact a number of water quality parameters. The National Urban Runoff Program (NURP) concluded that heavy metals (primarily copper, lead, and zinc), coliform bacteria, suspended solids, and nutrients (nitrogen and phosphorous) were the variables most influenced by urban runoff (2). Forest management activities (road construction, logging, slash burning) can increase soil erosion and thus sediment and turbidity (3), nitrate concentrations (4), and color (5). Agricultural land runoff can increase sediment, turbidity, dissolved solids, nutrients, bacteria, pathogenic organisms including cryptosporidium from feedlots and grazing areas, and herbicides and pesticides (6). Acid rain can result in elevated levels of mercury, aluminum, cadmium, lead, asbestos, and nitrates (7), particularly in small surface streams. While recreational uses of remote mountain streams increase the likelihood of *Giardia* contamination, cysts have been found even in very low use areas (8,9). Table 4.1.1 is a summary matrix of typical watershed land uses and the water quality variables they can affect, which was developed based on these and other references.

Typical Watershed Programs

Influence over these land use activities is highly dependent on the degree and method of control available to water utilities. Direct ownership of the land provides the most direct control over land use activities, but other avenues are available when the watershed is only partially owned, or not owned, by the utility. Written agreements with land owners, zoning ordinances, and various local, state, and federal planning and environmental laws and regulations all form the basis for regulating land use activities that may harm water quality. Buffer setbacks from streams and reservoirs, land use prohibitions, entry

Table 4.1.1. Land Use-Pollutant Analysis Matrix

Land Use Concern or Management Problem	Turbidity	Suspended Sediment	pH	Algae	Nitrogen	Phosphorous	Giardia	Cryptosporidium	Viruses
Cropland Runoff	×	×		×	×	×	×	×	×
Dairy Feedlots	×	×		×	×	×	×	×	×
Grazing	×	×		×	×	×	×	×	×
Recreation							×	×	×
Timber Management	×	×		×	×	×			
Road Construction	×	×		×	×	×			
Mining	×	×	×						
Industrial Discharge	×	×	×	×	×	×			
Sewage Discharge	×	×	×	×	×	×	×	×	×
Septic Tanks			×	×	×	×	×	×	×
Urbanization	×	×	×	×	×	×	×	×	×
Hazardous Waste Disposal									
Hazardous Waste Transport									
Acid Rain			×						

(Continued)

controls, large lot sizing, limitations on allowed impervious surface area on a site, utility approvals of facility designs, sanitary regulations, land acquisitions and exchanges, hazardous materials transportation restrictions, and special storm- and wastewater treatment facilities are all measures that can be taken in individual circumstances to control potential problems.

The literature describes examples of various watershed protection programs developed by water utilities. Burby *et al.* described an example of "downzoning," the use of large minimum lot sizes (anywhere from 1 to 10 acres depending on soils, slopes, proximity to streams, and other local conditions) to control development pressure, in Newark, New Jersey's watershed (1). The Scituate Watershed Study for Providence, Rhode Island, discussed methods for reducing risk from hazardous materials spills and other road runoff (10). Portland,

Table 4.1.1. (Continued)

Land Use Concern or Management Problem	Total Coliform	Fecal Coliform	THMs	Pesticides	VOCs	Other Synthetic Organics	Heavy Metals	Iron	Manganese
Cropland Runoff	×	×	×	×		×		×	×
Dairy Feedlots	×	×	×						
Grazing	×	×	×						
Recreation	×	×							
Timber Management	×	×	×	×				×	×
Road Construction	×							×	×
Mining							×	×	×
Industrial Discharge			×	×	×	×	×	×	×
Sewage Discharge	×	×	×	×	×	×	×	×	×
Septic Tanks	×	×	×	×	×				
Urbanization	×	×	×	×	×	×	×	×	×
Hazardous Waste Disposal				×	×	×	×		
Hazardous Waste Transport				×	×	×	×		
Acid Rain							×	×	×

Maine's use of septic system inspection was described by Grady (11). Controlling forest management activities in the Northwest was described for Seattle, Washington (12), and Portland, Oregon (13). A recently completed study funded by the American Water Works Association Research Foundation (AWWARF) documents those watershed control practices that have been found most effective across the country.

Monitoring and evaluating the effectiveness of the control measures can occur on several levels. Utilities can conduct water quality monitoring programs for parameters of concern in streams, reservoirs, and local potential contamination sources. Models for evaluating control options have been developed (15) and can be adjusted over time based on the observed results. The utility can also perform onsite inspections for conformance with any restrictions and can implement research programs to upgrade information on which decisions are based. The results of these activities must be periodically compared to the goals of the watershed protection program and to the list of control measures being used to determine whether the program is performing as desired and how it can be improved over time.

Directions in Watershed Protection Programs

The promulgation and implementation of EPA's Surface Water Treatment Rule, along with other environmental legislation, has and will have a significant impact on watershed management programs. Many watershed programs are being pushed from two somewhat contradictory directions; at the same time that watersheds feel pressures for increased use and activity, the requirements of legislation, regulation, and public expectations lead to a need for greater control and oversight of those activities. While the public is generally aware of the contamination potential from domestic and industrial waste point discharge sources, they are less aware of the subtle effects that increased urbanization, demands for recreational opportunities, and other lifestyle related activities can have on water quality. For example, development of homes in rural areas may result in increased contamination from sewage discharges if sewers are not available, septic tanks are improperly installed, or the capacity of the soil for septic systems is exceeded.

These problems raise difficult questions of equity concerning the distribution of costs that result from the need to control these activities and to clean up or provide treatment if control measures fail. A community may be forced to install expensive new water treatment equipment if watershed degradation occurs because of some uncontrolled land use, while the individual responsible for the use may not have to bear any cost. Conversely, a community may wish to impose significant expense on a land user to control a contamination source, when the land owner receives no benefit and may not even have had to worry about the contaminant if the community was not using the area as a watershed. As an example, a developer may be restricted to 5-acre lot size and be required to install stormwater retention ponds for sediment control in a watershed subdivision, while getting water supply from an entirely different water source.

These issues are also complicated by uncoordinated and conflicting environmental legislation and regulation at both the state and federal levels. Those with responsibilities for drinking water programs may not be aware of actions being taken by other agencies which will affect the land use and water quality that a watershed may produce. The reverse is also true. The following is one classic example. In the Surface Water Treatment Rule proposed in November 1987, EPA requires that suppliers of unfiltered water must have ownership or written agreements with landowners in the watershed. In a regulation proposed by the U.S. Forest Service in September 1987 concerning management of municipal watersheds, any new such formal agreements were specifically prohibited. Another example was a recent effort by the State of New York Department of Environmental Conservation to eliminate chlorination of wastewater treatment plant effluent as it is discharged into streams within the watershed of the City of New York, at the same time that the State Health Department was asking the city to provide stricter controls on sources of contamination in the watershed. To fully protect the nation's water resources, greater coordination will be needed between agencies responsible for Clean Water Act implementation, Safe Drinking Water Act implementation, ground-water protection measures, and land use planning with agencies responsible for watershed protection.

The role of the public in watershed issues is also increasing. Communities are no longer as willing to leave decisions about watershed management to government officials and technical experts. The public increasingly wants a say in the decision-making process through advisory committees, environmental impact statements, public hearings, and discussions with staff.

Portland's Watershed Protection Program

The watershed protection program for the Bull Run watershed, which serves as the primary water supply for Portland, Oregon, is a good example of the implementation of watershed protection issues and processes. This watershed is located about 25 miles east of Portland on the western slopes of the Cascade mountains. The physical drainage covers about 68,000 acres. About 95% of this land is owned by the federal government and administered along with a 30,000-acre buffer by the U.S. Forest Service as the Bull Run Management Unit. The watershed is dominated by Douglas Fir-Western Hemlock forests and ranges in elevation from 800 to 4,500 feet. Two manmade reservoirs and an enhanced natural lake serve as source water storage facilities for the City of Portland. Raw water turbidity averages about 0.4 NTU, with a typical range of 0.2 to just over 1 NTU. Monthly mean total coliform levels are below 15 plaque-forming units (PFU)/100 mL and 90th percentile levels are below 35 PFU/100 mL. Corresponding values for monthly fecal coliforms are 2 and 5 PFU/100 mL. Conductivity averages about 20 micromhos/cm, pH ranges from 6.8 to 7.4 units, and temperature from 0.5 to 15°C. The only treatment provided for the water is disinfection with chlorine and ammonia.

Management of the watershed is governed by special federal legislation (PL 95-200, 1977), which makes continued production of "pure clear raw potable water" the primary management goal and allows secondary uses compatible with that goal. The basic watershed management program is described in a Final Environmental Statement (FES) issued by the Forest Service for the watershed (16).

This FES was developed through a public process in which the Portland Water Bureau participated heavily.

The FES prohibits agricultural, recreational, and residential uses in the watershed. It allows timber harvesting where needed to protect water quality or where found to have no impact on it, and it prohibits pesticide use within the watershed boundaries. All road access points are gated and locks and keys changed every year. An Administrative and Operations Plan (17) has been developed to implement procedures and regulations on day-to-day activities such as road maintenance, reservoir operations, spill response, sanitation requirements for workers and contractors when in the watershed, and fire protection. All specific activities or projects that are not covered by these broader documents or that are undertaken to implement the general directives they provide are also assessed in an environmental planning process. As an example, while the FES generally calls for removing timber downed by windstorms due to the fire hazard it creates, the Forest Service also completed a separate Environmental Impact Statement (18) to specifically determine which acres were to be harvested after a severe 1983 storm.

In order to monitor management program performance, water quality standards have been developed at key stream locations in the watershed (19). These standards, based on historical water quality data, are used to identify and correct any degradation of water quality that may result from any management action. A network of about 20 longer term monitoring stations is supplemented by shorter term project monitoring stations centered around specific activities, to help interpret any changes in water quality detected by the standards. Physical and biological parameters such as turbidity, pH, conductivity, sediment, total and fecal coliforms, and HPC are monitored weekly at these sites. Nutrients such as nitrogen and phosphorous are examined every other week. Other parameters, such as chlorophyll a, metals, and pesticides are tested on schedules ranging from weekly to yearly depending on the location and parameter. Special studies, such as a several-year effort to document raw water *Giardia* cyst levels, are also conducted.

In addition to water monitoring, onsite field inspection of all approved activities takes place daily to ensure conformance to environmental plans. Natural resource inventories are conducted both before and after activities such as logging to ensure that the activity did in fact accomplish its intended results. Both water monitoring and field inspections provide feedback to the planning process to be used in improving future projects; reports describing these results are prepared and distributed to the public yearly.

Public involvement in the management program is heavy. A citizen advisory committee has been established and meets monthly to provide guidance to both the Forest Service and the City of Portland in reviewing watershed management plans and policies. Special open public meetings are held to discuss particular management issues of concern. Watershed tours are arranged for interested parties. The Portland Water Bureau prepares and distributes public information summaries and materials on water quality and watershed management issues. It also works closely with local media to ensure that information on activities is distributed throughout the community.

The major issues within the Bull Run watershed are related: the impacts of logging activities on water quality and the long-term relationship between human watershed management activities and natural catastrophes such as wind, fire, and flood. The watershed program must balance the risks between acting and failing to act. It must look at whether conducting "management" such as logging will improve or harm the watershed in both the short and long term and whether short- and long-term risks are worth the benefits.

In order to better answer these questions, research, studies, and operational improvements are continually being made in the watershed program. Recently, experimental remote turbidity monitoring stations have been installed, a special study on sediment sources and transport in the watershed was conducted, an independent peer review of the watershed monitoring program was commissioned, and a study was conducted to quantify the levels of *Giardia* cysts at the raw water intake.

The Portland Water Bureau works closely with the County Health Department to track giardiasis in the community (giardiasis has been a reportable disease in Oregon since 1982). A study was recently completed comparing the observed risks of getting giardiasis in Portland with those in a nearby similar filtered water community (20). No evidence of waterborne transmission of giardiasis in Portland's unfiltered supply was detectable from the epidemiological data.

Being responsive to the public concerns about the watershed includes continually assessing the public health impact of the watershed and water treatment programs. The results from these and similar activities will be used to ensure that the water quality produced by the watershed continues to be the best possible and continues to meet all requirements of use as an unfiltered, disinfected supply.

Conclusions

An effective watershed management program depends upon a conscious decision-making process. This process involves understanding and characterizing the watershed, identifying the goals of watershed management and the means of achieving those goals, and monitoring the effectiveness of protection measures to provide feedback for improving the watershed program. A process such as this is likely to identify the land uses and activities which have been found to impact water quality and the measures found effective at controlling them elsewhere. Portland's watershed protection program for the Bull Run watershed is an example of a successful program that meets its stated goal of providing "pure, clear, raw, potable water."

Watersheds across the country are facing increased pressure from urbanization, demand for recreational opportunities, and other access to the resources they contain. Combined with stricter federal and state drinking water regulations such as the Surface Water Treatment Rule and other environmental regulations, these pressures make the need for better watershed management programs critical. As the number of contaminants a water supplier must be concerned about increases, the risks the public is willing to accept decrease, and the cost and complexity of advanced treatment processes required for these contaminants increase, watershed protection becomes more essential for all water suppliers. In order to meet these challenges, federal and state agencies with responsibilities for activities that can impact watersheds must develop greater coordination, and must encourage more effective public involvement.

References

1. Burby, R.J., Kaiser, E.J., Miller, T.L., and Moreau, D.H. Drinking Water Supplies Protection through Watershed Management. Ann Arbor Science Publishers, Ann Arbor, Michigan, 1983.

2. U.S. Environmental Protection Agency. Results of the Nationwide Urban Runoff Program, Volume I - Final Report. Washington, D. C., 1983.

3. Beschta, R.L. Long-term patterns of sediment production following road construction and logging in the Oregon coast range. *Water Resources Research.* 14(6): 1011, 1978.

4. Pierce, R.S., Martin, C.W., Reeves, G.F., Likens, G.E., and Borman, F.H. Nutrient loss from clearcutting in New Hampshire. *In:* Proceedings of a Symposium on Watersheds in Transition, American Water Resources Assoc., 1972.

5. Taylor, R.L., Adams, P.W., Nelson, P.O., and Seidler, R.J. Effect of Hardwood Leaf Litter on Water Quality and Treatment in a Western Oregon Municipal Watershed. Oregon State University Water Resources Research Institute, WRRI- 82, 1983.

6. Christensen, L.A. Water Quality: a Multi-disciplinary Perspective, in Problems and Potentials for Agricultural Communities. *American Water Resources Association.* 1984.

7. Quinn, S.O. and Bloomfield, N. (eds.) Proceedings of a Workshop on Acidic Deposition, Trace Contaminants and their Indirect

Human Health Effects: Research Needs. EPA Environmental Research Laboratory, Corvallis, Oregon, 1985.

8. Suk, T.J., Sorenson, S.K. and Dileanis, P.D. The relation between human presence and occurrence of *Giardia* cysts in streams in the Sierra Nevada, California. *J. Freshwater Ecology*. 4(1):71, 1987.

9. Ongerth, H.J. Public health viewpoint, AWWA Joint Discussion: Watershed Management and Reservoir Use. *J. AWWA*. 56(2) 149, 1964.

10. Joubert, L.B. Watershed protection strategies for the Scituate reservoir. *J. New England Water Works Assoc*. 99(2):136, 1985.

11. Grady, Robert P. Source protection of a multipurpose lake: a utility's perspective. *In:* R.B. Pojasek (ed.). Drinking Water Enhancement through Source Protection. Ann Arbor Science Publishers, Ann Arbor, Michigan, 1977.

12. Monahan, J.E. and Courchene, J.E. Seattle's watershed protection and monitoring program. *In:* 1986 AWWA Water Quality Technology Conference Proceedings, Portland, Oregon, 1977.

13. Robbins, R. Development and application of water quality standards for the Bull Run watershed. *In*: Proceedings of the Water Quality Technology Conference - 14. AWWA, November 1986.

14. Robbins, R.W., Glocker, J.L., Bloem, D.M., and Niss, B.M. Effective Watershed Management. American Water Works Association Research Foundation (in Press).

15. Donigian, A.S. and Rao, P.S. Selection, application, and validation of environmental models. International Symposium on Water Quality Modeling of Agricultural Nonpoint Sources, Logan, Utah, 1988.

16. U.S. Forest Service. Bull Run Planning Unit Final Environmental Statement. Mt. Hood National Forest, 1979.

17. U.S. Forest Service. Bull Run Watershed Administration and Operation Plan. Mt. Hood National Forest, 1984.

18. U.S. Forest Service. Bull Run Blowdown Final Environmental Impact Statement. Mt. Hood National Forest, 1987.

19. U.S. Forest Service. Water Quality Standards for Bull Run Watershed Management Unit. Mt. Hood National Forest, 1987.

20. Glicker, J. and Edwards, R. The risk of endemic giardiasis from an unfiltered protected water source. *In:* Proceedings of the Water Quality Technology Conference - 15. AWWA, 1987.

Principles of Water Filtration

by: Gary S. Logsdon
Director, Water Process Research
Black & Veatch Engineers-Architects
1025 Alliance Road, Suite 101
Cincinnati, Ohio 45242
(513) 984-6630

[At the time this article was written, Dr. Logsdon was with
the Risk Reduction Engineering Laboratory,
U.S. Environmental Protection Agency]

Introduction

Three distinctly different filtration processes are generally used by water utilities in the United States – slow sand filtration, diatomaceous earth filtration, and coagulation followed by rapid rate filtration. An understanding of the similarities and differences of each of the three processes would be enhanced by knowledge of principles related to colloid stability and particle removal in a packed bed. This article, therefore, presents a brief review of filtration principles, followed by a discussion of the capabilities of the three kinds of filtration processes.

Colloid Stability and Coagulation

Many of the particles that need to be removed by filtration are in the colloidal size range 0.001 to 0.1 µm (1). Colloidal particles possess a surface electrical charge, typically a negative charge for the colloids of concern in water filtration. Information on the origin and nature of such charges is given by Sawyer and McCarty (1). Because of their surface charge, colloids tend to remain in suspension and are said to be stable. Positively charged inorganic coagulants or polymers are used to overcome the effects of the negative surface charges of colloids, destabilizing them, so that they will agglomerate and slowly settle to the bottom of sedimentation basins or stick to grains of filtering material in the filtration process. It is absolutely essential that colloids be destabilized if rapid rate (rapid sand or multi-media) filtration is used, because stable (uncoagulated) colloidal suspensions cannot be filtered effectively by these filters.

Use of the correct dose of coagulant is important. Dose control can be accomplished in a variety of ways. One traditional approach is jar testing. For waters of perhaps 10 NTU or higher, jar testing combined with continuous monitoring of the turbidity of the filtered water at individual filters is an approach frequently used. If raw water quality can change rapidly or the raw water turbidity is low (below 10 NTU), jar tests may not be very effective, because of the time required for testing or the smaller differences in raw and settled water turbidities. In such instances, coagulant dose control by zeta potential instrumentation, a streaming current detector, or a pilot filter may be appropriate. Wagner and Hudson suggested that filtration using Whatman No. 40 paper could give information on the treatment levels that produce acceptable water quality (2). The chemical dosing requirements for successful treatment of a raw water are not constant. It is essential that testing be done periodically to determine the optimum conditions for coagulation; this is often not done, especially at small plants.

Particle Removal in a Packed Bed

Fundamentals of granular media filtration have been studied by numerous researchers in recent decades. A review of much of this work was recently published in the *Journal of the American Water Works Association* (3). An excellent description of principles of particle removal by individual collector grains and by a packed bed of collector grains (a filter bed) was given in *Water Treatment Principles and Design* (4). The following discussion of particle removal in a packed bed is based on this information.

Removal of particles by three different mechanisms is shown in Figure 4.2.1, which depicts a single collector grain. The four solid lines labeled A, B, C, and D represent streamlines in a laminar flow condition. Unless acted upon by outside forces, small particles will follow the streamlines. The small particle (less than 1 µm) is acted upon by water molecules and moves in a random fashion (Brownian movement). Brownian movement can cause the small particle to deviate from the streamline and may even cause it to collide with the collector grain. The equation for particle removal efficacy by Brownian movement (η_B) is given in Figure 4.2.2. The particle in streamline B is removed by sedimentation. Because it is more dense than water, this particle's motion is influenced by gravity as the streamline curves around the collector grain. Sedimentation causes the particle to deviate from the streamline. The equation for efficiency of removal by sedimentation (η_s) is also given in Figure 4.2.2. In streamline C, a large particle moves along the streamline. It is sufficiently large that it touches the collector grain and is removed by interception. Figure 4.2.2 gives the equation for interception efficiency (η_I) as well. The overall efficiency of a single collector is the sum of the efficiencies for the various removal mechanisms (Figure 4.2.3).

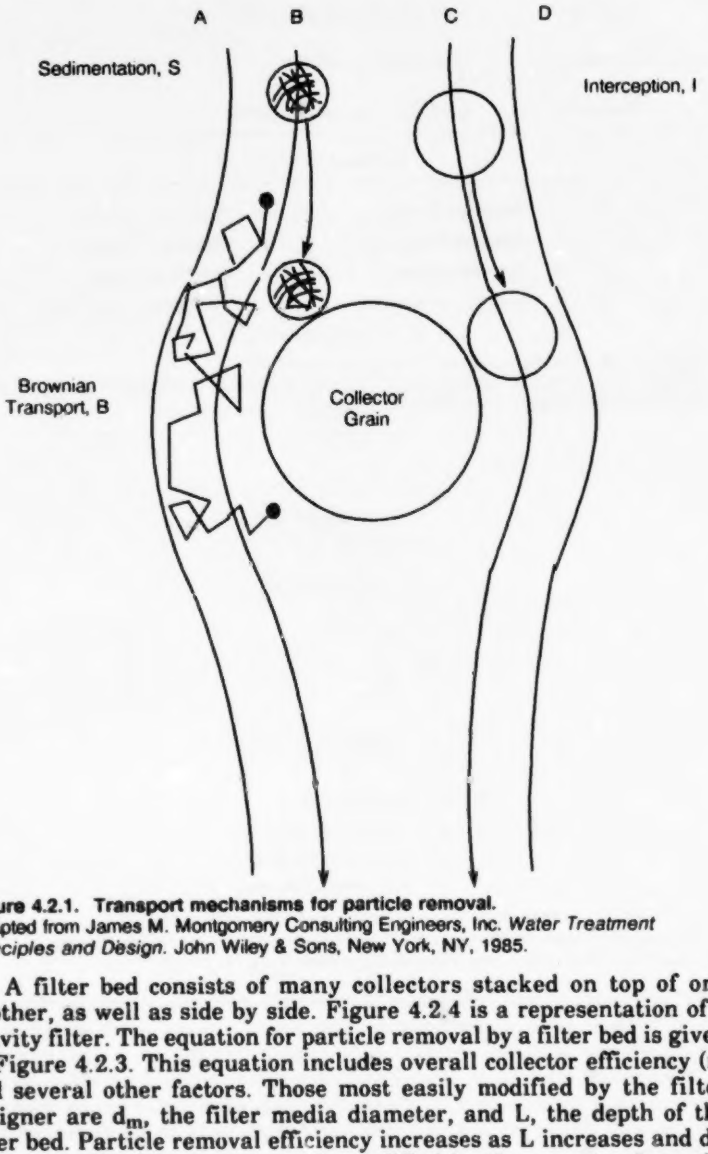


Figure 4.2.1. Transport mechanisms for particle removal.
Adapted from James M. Montgomery Consulting Engineers, Inc. *Water Treatment Principles and Design*. John Wiley & Sons, New York, NY, 1985.

A filter bed consists of many collectors stacked on top of one another, as well as side by side. Figure 4.2.4 is a representation of a gravity filter. The equation for particle removal by a filter bed is given in Figure 4.2.3. This equation includes overall collector efficiency (η) and several other factors. Those most easily modified by the filter designer are d_m, the filter media diameter, and L, the depth of the filter bed. Particle removal efficiency increases as L increases and d_m decreases. The factor most easily modified by the operator is α, the

Brownian $\eta_B = 0.9\,[kT/\mu\, d_p d_m V_o]^{2/3}$

Interception $. = 1.5\,(d_p d_m)^2$

Sedimentation $\eta_s = [(\rho_p - \rho_w)\, g d_p^2]/18\mu V_o$

Definition of Terms

K = Boltzman Constant Vo = Approach Velocity
T = Absolute Temperature ρ_p = Density of Particle
μ = Dynamic Viscosity ρ_w = Density of Water
d_p = Diameter of Particle g = Acceleration of Gravity
d_m = Diameter of Media

Figure 4.2.2. Single collector efficiency equations.
Adapted from James M. Montgomery Consulting Engineers, Inc. *Water Treatment Principles and Design*. John Wiley & Sons, New York, NY, 1985.

Collector Efficiency
$\eta = \eta_B + \eta_I + \eta_s$

Filter Efficiency

$$\frac{C}{C_o} = \exp\left[\frac{-\Psi\,(1 - \varepsilon_o)}{d_m}\;\alpha L \eta\right]$$

Definition of Terms

Ψ = Shape Factor
ε_o = Porosity of Clean Bed
L = Depth of Filter Bed
α = Attachment Efficiency

Figure 4.2.3. Overall collector efficiency and filter efficiency.
Adapted from James M. Montgomery Consulting Engineers, Inc. *Water Treatment Principles and Design*. John Wiley & Sons, New York, NY, 1985.

particle attachment efficiency. For stable colloids, α would approach zero, indicating that no colloids that touch the collector grain would stick. For destabilized colloids, if 100 percent of the particles that touch the collector stick to it, $\alpha = 1.0$. Coagulation should be optimized, so that α can approach 1.0. Particle size also influences removal efficiency. Particles in the 1 µm size range tend to be removed least efficiently.

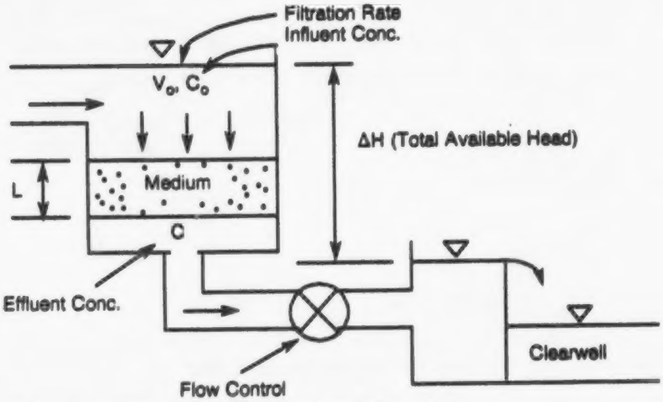


Figure 4.2.4. Gravity filtration through granular media.

Removal of very large agglomerations of particles is accomplished with yet another method. For any packed bed of collector grains, there exists a limit on the size of particle that can pass through the pores. Particles that are too large to pass through a packed bed are removed by the mechanism of straining. Very large floc particles are removed in this way in sand filters. *Giardia* cysts are removed by straining in the diatomaceous earth filtration process. In slow sand filtration and in rapid rate filtration, *Giardia* removal occurs by the mechanisms described above for particle removal in a packed bed. In slow sand filters, larger organisms may prey upon *Giardia* cysts for a food source and accomplish removal in this fashion also.

A summary of factors influencing particle removal efficiency is given in Tables 1 and 2. Attachment efficiency α plays a critical role in particle removal. Control of coagulation chemistry to maximize α is essential for effective operation of coagulation-filtration plants. Particle density, ρ_p, and concentration of particles in raw or influent water, C_o, can be modified to a limited extent. Flocculation energy can influence ρ_p, as more intense flocculation can promote formation of denser flocs. Effective sedimentation can reduce particle concentration by perhaps one order of magnitude, and should thus result in a reduced concentration of particles in the filter effluent. Filtration rate is a function of both design and operation. Higher filtration rates reduce particle removal by Brownian movement and sedimentation according to the equations in Figure 4.2.2. In addition, the larger shear forces associated with higher filtration rates would tend to remove a larger fraction of particles that did touch and attach to collector grains, including those removed by interception. Decreasing the filter media size creates more surfaces for attachment and places more collectors in the same bed depth, increasing particle removal efficiency. Increasing filter bed depth increases the number of collectors a particle must successfully pass in order to reach the effluent. Thus deeper beds are more effective for particle removal, all other factors being equal.

Table 4.2.1. Factors Influencing Particle Removal Efficiency

Parameter	Change in Parameter	Change in Effluent Concentration
Attachment Efficiency, α (0.05 - 1.0)	↑	↓
Particle Density, ρ_p (1.02 - 2.5 g/cm^3)	↑	↓
Influent Particle Concentration, C_o (< 1 - 100 mg/L)	↑	↑

Adapted from R.D. Letterman, private communication, 1988.

Table 4.2.2. Design Factors Influencing Particle Removal Efficiency

Parameter	Change in Parameter	Change in Effluent Concentration
Filtration Rate, V_o (0.04 - 1.0 gpm/ft^2)	↑	↑
Medium Grain Diameter, d_m (0.3 - 3.0 mm)	↑	↑
Bed Depth, L (1 - 8 ft)	↑	↓

Adapted from R.D. Letterman, private communication, 1988.

Filter run length is also an important concern. Efficient plant operation requires runs of sufficient length so that most of the water produced can be used for the needs of the community. When runs are very short, a substantial portion of treated water has to be used to backwash filters. This is inefficient and wasteful of both water and plant labor.

Water Treatment Principles and Design lists factors that influence filter run length (Table 4.2.3) (4). Increasing filter media diameter lengthens the time to maximum head loss by enabling the filter to store large floc particles. Large media grains are less efficient as collectors, however, so turbidity breakthrough could occur faster with larger media. Because deeper filter beds are more effective particle collectors, they lengthen the time to turbidity breakthrough, but deeper beds also have higher clean bed head loss and thus reach maximum head loss sooner. Higher filtration rates push particles through and out of filter beds faster and cause higher head loss. Both effects reduce run length. Higher influent particle concentration raises the effluent concentration, causing the filter to clog sooner. Stronger floc adheres in the pore spaces of the filter bed more effectively, thereby increasing time to turbidity breakthrough. On the other hand, stronger floc can increase head loss or even "blind" a filter. Higher porosity in the filter bed provides passages for floc particles through the bed and decreases time to turbidity breakthrough. Greater porosity, by providing more floc storage volume, increases time to maximum head loss. Denser deposits of floc in the filter bed result in a slower decrease in the porosity of the bed as floc is removed. This hastens turbidity breakthrough but permits storage of more floc, thus reducing the rate of head loss buildup. Table 4.2.3 shows that numerous conflicting circumstances must be dealt with in both the design and operation of filters. Balancing of various factors and achieving suitable tradeoffs are necessary for successful design and operation of water filters.

Table 4.2.3. Design Factors Influencing Particle Removal Efficiency

	Effect of Parameter Increase	
Parameter Increased	Time to Turbidity Breakthrough	Time to Maximum Head Loss
d_m	↓	↑
L	↑	↓
Q/A (V_o)	↓	↓
C_o	↓	↓
Floc Strength	↑	↓
Porosity (ε)	↓	↑
Deposit Density	↓	↑

Adapted from James M. Montgomery Consulting Engineers, Inc. *Water Treatment Principles and Design*. John Wiley & Sons, New York, NY, 1985.

Regardless of the type of process used, filtration tends to be performed on a batch rather than a continuous basis. All filters, if operated for a sufficiently long time, will remove enough particulate matter from the influent water to change the hydraulic characteristics of the filter. As more and more particles are stored in the filter, progressive clogging occurs. This clogging can eventually cause the head loss across the filter to increase to a maximum operating value. A clogged, or dirty, filter must be removed from service, cleaned, and prepared for service again. Various filtration processes differ in the frequency and nature of the cleaning step. This is explained in the following sections.

Slow Sand Filtration: Principles and Capabilities

Slow sand filtration originated in Great Britain in the 1800s. In this process, uncoagulated water is applied to a sand bed about 1 meter deep at a filtration rate (approach velocity) of about 0.1 meter/hour (m/hr.). Removal of microorganisms is aided by biological processes that occur on and in the filter bed, where an ecosystem becomes established with extended use. On the top of the media, a biologically active scum layer (schmutzdecke) builds up and assists in filtration. As the water enters the schmutzdecke, biological action breaks down some organic matter, and inert suspended particles may be physically strained out of the water. The water then enters the top layer of sand where more physical straining and biological action occur and attachment of particles onto the sand grain surfaces takes place. Also some sedimentation may occur in the pores between the media grains where velocity is sufficiently slow. In a slow sand filter, the efficiency of particle attachment (α) may be enhanced by production of natural polymers by the biota living in the schmutzdecke and in the filter bed.

Slow sand filter maintenance generally is not complicated or time consuming, and can be done in times as short as 1 hour at small slow sand filtration plants. Filter cleaning, however, is somewhat involved. When the depth of the schmutzdecke layer increases, clogging the top sand layer and causing the head loss through the filter to reach a predetermined level, the filter must be drained and the top layer of sand removed. This period of scraping is the only time during normal operations in which additional manpower may be required to operate the filter. The amount of time between scrapings will depend on how much material is filtered out of the water. Thus high turbidity levels would shorten the cycle. Slow sand filter runs can be as short as 1 or 2 weeks when muddy or algae-laden waters are treated. The runs can last for several months when very clear waters are filtered.

Slow sand filters are uncomplicated and easy to operate, but they can be used successfully only with a good quality of raw water (turbidity usually less than 10 NTU and no undesirable inorganic or organic chemicals present). In comparison to other filtration processes, they also require large land areas per volume of water treated, and thus would probably not be used by large water utilities to the United States. Slow sand filtration is a very effective treatment process for control of microorganisms, however, when the raw water quality is appropriate.

Slow sand filters have been shown capable of removing 99 to 99.99 percent of the raw *Giardia* cysts in water (5,6,7). Using pilot filters Bellamy *et al.* found that cyst removal did not deteriorate after filter scraping (5). Pyper observed that at 7.5°C to 21°C, cyst removal was 99.98 percent to 99.99 percent. At 0.5°C to 0.75°C, removal ranged from 99.36 percent to 99.91 percent; however, at 0.5°C, cyst removal deteriorated to 93.7 percent when both *Giardia* cysts and primary unchlorinated sewage effluent were added to the raw water simultaneously. In this situation, the loading of organisms in the influent water may have been greater than the established biological population of the slow sand filter could cope with.

Schuler *et al.* studied the removal of *Cryptosporidium* oocysts and found that a pilot-scale slow sand filter was at least as effective for removal of *Cryptosporidium* oocysts as for *Giardia* cysts (8). Removal of *Cryptosporidium* was 99.98 percent or greater.

Total coliform removal was found to be adversely influenced by increases in filtration rate from 0.04 to 0.4 m/hr (5), by decreases in filter bed depth from 0.97 to 0.48 m (6), by increases in sand size from 0.13 mm to 0.61 mm and by decreases in temperature from 17°C to 2°C (6). Of these parameters, the 0.61-mm sand size would be greater than sizes typically used in slow sand filters and might have accentuated the adverse impact of that variable. The use of 0.61-mm sand resulted in average total coliform removal of 96 percent versus 99.4 percent for 0.13-mm sand. Temperature decreases from 17°C to 5°C or 2°C resulted in deterioration in coliform removal from the 99 percent level to about 90 percent for the colder waters. Cleasby *et al.* found that total coliform removal was lower during the first 2 days after scraping than during the remainder of the run (9). In some instances, differences in the two time periods were slight, but five of nine runs exhibited coliform removals ranging from 82 to 95 percent during the first 2 days. During the remainder of the runs, removals ranged from 97 to 100 percent.

Virus removal has been reported to be influenced by both temperature and filtration rate (10). At 5 to 8°C, poliovirus removal was 98.25 percent at a filtration rate of 0.5 m/hr, but was 99.68 percent at a rate of 0.2 m/hr . At 16 to 18°C, poliovirus removal was higher, ranging from 99.865 percent at 0.4 m/hr. to 99.975 percent at 0.2 m/hr. These encouraging results suggest that slow sand filtration would be effective against a broad spectrum of microbiological contaminants.

Diatomaceous Earth Filtration: Principles and Capabilities

During World War II, an intense effort was made to develop a filtration process that would achieve practically complete removal of *E. histolytica* cysts. The result was the application of the diatomaceous earth (DE) filtration process to potable water treatment. In DE filtration, a thin coating (3 to 5 mm) of diatomaceous earth is placed on a filter septum by recirculating a slurry of DE through the filter until essentially all of the DE is on the filter septum. After the filter cake is established in the precoating process, raw water that has been dosed with a small amount of DE is passed through the filter. Particles are removed, and they accumulate on the filter cake. Because DE (the body feed) is added to the raw water, the good hydraulic characteristics of the filter cake are maintained, and long filter runs can be attained.

When a run is terminated, the filter cake is removed, disposed of, and fresh DE is used to recoat the clean septum.

In DE filtration, mechanisms of particle removal depend on particle size. Walton presented information, including photomicrographs, that showed that DE filters remove *Giardia* cysts by a straining or screening action (11). Because of the size of these cysts, their passage through the DE filter cake is physically blocked. On the other hand, removal of bacteria could occur by straining when very fine grades of DE are used, or it could occur by means of surface attachment when coarse DE grades are coated with positively charged aluminum or iron precipitates or cationic polymers. Virus removal would occur primarily by surface attachment, regardless of the grade of DE being used, as long as the viruses were not attached to larger particles in the raw water.

Diatomaceous earth filters have been studied for removal of a variety of contaminants and have been shown to attain excellent removal of *Giardia* cysts over a broad range of operating conditions. Cyst removal exceeding 99 percent, and often 99.9 percent, were reported by Lange *et al.* (12) for filtration rates of 2.4 to 9.6 m/hr, for temperatures from 3.5 to 15°C, and for four different grades of diatomaceous earth (Celite 545®, Celite 535®, Celite 503®, and Hyflo Super-Cel®). Logsdon *et al.* reported that when sufficient DE precoat and body feed were used, removal of 9µm radioactive beads was nearly always 99.9 percent or higher (13). Use of a precoat of at least 1.0 kg/m^2 was shown to be appropriate for obtaining most effective removal of the 9µm particles. They also reported that 11 filter runs were made with *G. muris* cysts at filtration rates of 2.2 to 3.5 m/hr, with Celite 535® precoat and body feed. Cyst removal exceeded 99.0 percent in all runs, and exceeded 99.9 percent in five of the runs. DeWalle *et al.* reported on four DE filter runs conducted for *Giardia* cyst removal (14). Cyst removal exceeded 99 percent in each of the four runs. The overall results of all research for *Giardia* cyst removal indicate that DE filtration is very effective for controlling *Giardia* cysts. Factors important to continued effective performance are using adequate precoat and body feed and keeping the septum very clean (good cleaning at the end of each run).

Schuler *et al.* reported on an evaluation of DE filtration for removal of both *Giardia* cysts and *Cryptosporidium* oocysts (8). In four of five runs, no *Giardia* cysts were recovered in the filtered water. *Cryptosporidium* results were not quite as good, but removal exceeded 99.99 percent in all runs. Removal of total coliform bacteria by DE filtration was studied extensively at Colorado State University by Lange *et al.*(12). Coliform removals were strongly influenced by the grade of diatomaceous earth used. Coarser grades attained removals ranging from 30 to 50 percent for Celite 545®, 50 to 70 percent for Celite 503®, and 92 to 96 percent for Celite 512®. Total coliform removal with Super-Cel® was 99.92 percent to greater than 99.98 percent.

Brown *et al.* reported that a high percentage of removal could be attained for poliovirus when coated DE filter aid was used or when cationic polymer was added to the raw water (15). In one 12-hour filter run, diatomaceous earth coated with 1 mg of cationic polymer per gram of DE produced filter water in which no viruses were recovered from 11 samples (removal >99.95 percent). In one instance, in which 1 of 12 samples was positive, virus removal was 99 percent. In a 12-hour run in which uncoated DE was used and 0.14 mg/L of cationic polymer was added to the raw water, no viruses were recovered from any of the 12 samples analyzed.

Work was done at Colorado State University to improve the capabilities of DE filtration. In order to alter the surface properties of diatomaceous earth, aluminum hydroxide was precipitated to the surface of a DE slurry. With 0.05 grams of alum per gram of Celite 545®, total coliform removal was 99.86 percent, as compared to 30 to 50 percent removal for uncoated Celite 545®. For the same grade of DE, turbidity removal was 98 percent for coated DE versus less than 20 percent for uncoated DE (12). These results show that the straining mechanism of removal can be augmented by a surface attachment removal mechanism if DE is given an electropositive coating.

Diatomaceous earth filtration, like slow sand filtration, is most appropriate for use with high quality raw water. Limits on the quality of raw water that would be appropriate for DE filtration are not easy to set. The process removes particulate matter by trapping it within the filter cake. As the concentration of particulate matter in raw water increases, the load applied to the filter cake increases. To maintain high permeability of the filter cake and good head loss characteristics, body feed diatomaceous earth is added to the raw water. A rule of thumb is that higher raw water particle concentrations require more body feed, if the nature of the particles does not change. The nature, and especially the compressibility, of the particles being removed is quite important. Rigid turbidity-causing particles, such as very fine sand, would not block or blind the filter cake, but compressible particles, such as algae, coagulation floc, precipitated iron, or biological matter could. Pilot filtration studies are advisable if the water in question is not already being treated by DE filtration. Such studies would establish the appropriate grade of DE to ensure the desired effluent turbidity, the amount of body feed to add under conditions of the test runs, and the approximate length of filter run to expect.

Coagulation-Filtration: Principles and Capabilities

Near the turn of the century, engineers in the United States began to recommend the use of rapid sand filters for treatment of muddy surface waters like those found in the Ohio River and Mississippi River valleys. These filters treat water that has been conditioned by coagulation, flocculation, and sedimentation to remove most of the particulate matter; this results in longer filter runs. Rapid sand filters typically were operated at rates of 5 m/hr in the first half of this century. Since about 1960, use of dual media (coal and sand) or mixed media (coal, sand, and garnet or ilmenite) has permitted operation of rapid rate filters at rates as high as 10 to 24 m/hr. For clear waters (10 NTU or lower) the sedimentation and sometimes flocculation processes may be omitted, and the process is known as direct filtration. In direct filtration, all removal of particulate matter occurs within the filter.

Effective coagulation is essential for successful operation of rapid rate filters, with or without sedimentation, because surface attachment is the mechanism by which particle removal occurs. The principles of colloid destabilization and attachment onto collector grains described earlier in this paper are especially important for coagulation-filtration. Unless the coagulation step is properly managed, this process cannot attain the high degree of particle removal of which it is capable. When the raw water is not coagulated at all, or is not coagulated properly, coagulation-filtration simply cannot attain maximum particle removal on a consistent basis.

After a rapid rate filter has been in service for a period of time, filtration is stopped and the filter bed is cleaned by backwashing. Typically surface wash is also used to supplement the cleaning action of the backwash water. Some newer installations employ an air scour step to enhance the cleaning of media. Backwashing needs to be done carefully so that the filter bed is thoroughly cleaned but not unduly disturbed by the backwashing procedure. Careless handling of water or air during the backwashing could even result in damage to the filter bed and the media support system.

Most U.S. coagulation-filtration research for *Giardia* cyst removal has focused on the coagulation-filtration (in-line) or coagulation-flocculation- filtration (direct filtration) variations of the process, because waterborne giardiasis outbreaks tended to be observed in regions of the country that had low turbidity waters, which were thought to be suitable for such treatment. Research by Logsdon *et al.* (13), DeWalle *et al.* (14), and Al-Ani *et al.* (16) involved coagulation with alum, or alum plus a polymer; filtration through sand or dual media at 5 to 14 meters/hr; and temperatures ranging from 3 to 20°C. Later research (17) was conducted on conventional treatment, with alum or alum and polymer, dual media, and three monomedia types (sand, anthracite, GAC); filtration at 7 m/hr; and at room temperature (about 25°C).

Results of the three cited direct filtration studies indicate that *Giardia* cyst removal can exceed 99.0 percent or even 99.9 percent when the raw water is coagulated properly and filtered. Results of

Logsdon *et al.* (1981) and DeWalle *et al.* (1984) indicated that with proper pretreatment, cyst removal exceeded 99.0 percent when filtered water turbidity was below 0.30 NTU. Al-Ani *et al.* showed that cyst removal of 99 percent or more was likely to occur if turbidity removal was 70 percent or more, when raw waters in the 0.2 to 1 NTU range were treated (16). This would produce filtered waters in the 0.06 to 0.30 NTU range.

All of the above researchers showed that dependable cyst removal results can be attained if a clear water (about 1 NTU) is filtered without being properly coagulated. Use of no coagulant, or of an improper dose, resulted in erratic cyst removal results. In addition, DeWalle *et al.* showed that for alum coagulation, proper pH is necessary when testing soft, low alkalinity water (14). They observed effective treatment at pH 5.6 and 6.2, but at pH 6.8 with alum coagulation, cyst removal was reduced from 99 to 95 percent.

The coagulation-filtration process can remove a variety of contaminants. Robeck *et al.* showed that direct filtration could remove 90 percent to 99 percent of viruses, while conventional treatment removals consistently were 99 percent (18). McCormick and King stated that coliform removal by direct filtration was practically 100 percent when filtered water turbidity was 0.10 NTU or less (19).

Coagulation and filtration can be used to treat a very wide range of raw water quality. Turbidities in the range of hundreds of NTU can be treated when sedimentation is used. Water utilities along the Missouri River sometimes employ plain sedimentation or chemically assisted sedimentation ahead of a conventional treatment train. Such plants can handle raw water of 1,000 NTU or more.

An important consideration at all plants employing coagulation is that the level of operating skill needed is substantial. In order to effectively and efficiently control the coagulation-filtration process and attain low filtered water turbidity, operators need to understand the chemical aspects of coagulation, regardless of plant size. Large and medium-sized plants are able to hire and keep trained operators who can effectively operate coagulation-filtration plants. Small plants, however, may not have the resources to hire or train operators who have a solid understanding of coagulation. This can lead to problems of poor treated water quality, if operators are unable to adjust treatment when raw water quality changes.

Summary

1. The mechanisms by which particles are removed in packed beds are understood to an extent sufficient for developing general concepts of filter design and operation.
2. Each of the three filtration processes reviewed is different, and no single process is ideal in every circumstance.

3. As process complexity increases, from slow sand filtration to DE filtration, to coagulation-filtration, the skill level needed for effective operation increases, and producing high quality filtered water increasingly becomes dependent on operator skill and ability.

4. When properly designed and operated, the filtration processes reviewed can substantially reduce the concentration of microorganisms in drinking water.

References

1. Sawyer, C.N. and McCarty, P.L. Chemistry for Environmental Engineering, Third Edition. McGraw-Hill, New York, N.Y. 1978.
2. Wagner, E.G. and Hudson, H.E., Jr. Low-dosage high-rate direct filtration. *J. AWWA*, 74:5:256-261, 1982.
3. Amirtharajah, A. Some theoretical and conceptual views of filtration. *J. AWWA*, 80:12:36-46, 1988.
4. James M. Montgomery Consulting Engineers, Inc. Water Treatment Principles and Design. John Wiley & Sons, New York, N.Y., 1985.
5. Bellamy, W.D., Hendricks, D.W., and Logsdon, G.S. Slow sand filtration: influences of selected process variables. *J. AWWA*, 77:12:62-66, 1985.
6. Bellamy, W.D., Silverman, G.P., and Hendricks, D.W. Filtration of *Giardia* cysts and other substances: Volume 2. Slow Sand Filtration. Report No. EPA 600/2-85/026, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1985.
7. Pyper, G.R. Slow sand filter and package treatment plant evaluation: operating costs and removal of bacteria, *Giardia*, and trihalomethanes. Report No. EPA/600/2-85/052, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1985.
8. Schuler, P.F., Ghosh, M.M. and Boutros, S.N. Comparing the removal of *Giardia* and *Cryptosporidium* using slow sand and diatomaceous earth filtration. *In*: Proceedings 1988 AWWA Annual Conference, pp. 789-805, 1988.
9. Cleasby, J.L., Hilmoe, D.J., and Dimitracopoulos, C.J. Slow sand and direct in-line filtration of a surface water. *J. AWWA*, 76:12:44-55, 1984.
10. Poynter, S.F.B. and Slade, J.W. The removal of viruses by slow sand filtration. *Prog. in Water Tech.*, 9:1:75-88, 1977.
11. Walton, H.W. Diatomite filtration: how it removes *Giardia* from water. *In:* Wallis, P.M. and Hammond, B.R. (eds.), Advances in

Giardia Research, Univ. of Calgary Press, Calgary, Alberta, pp. 113-117, 1988.

12. Lange, K.P., Bellamy, W.D., Hendricks, D.W., and Logsdon, G.S. Diatomaceous earth filtration of *Giardia* cysts and other substances. *J. AWWA*, 78:1:76-84, 1986.

13. Logsdon, G.S., Symons, J.M., Hoye, R.L., Jr., and Arozarena, M.M. Alternative filtration methods for removal of *Giardia* cysts and cyst models. *J. AWWA*, 73:2:111-118, 1981.

14. DeWalle, F.B., Engeset, J., and Lawrence, W. Removal of *Giardia lamblia* cysts by drinking water treatment plants. Report No. EPA-600/2-84-069, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1984.

15. Brown, T.S., Malina, J.F., Jr. and Moore, B.D. Virus removal by diatomaceous earth filtration - Part 2. *J. AWWA*, 66:12:735-738, 1974.

16. Al-Ani, M.Y., Hendricks, D.W., Logsdon, G.S., and Hibler, C.P. Removing *Giardia* cysts from low turbidity waters by rapid rate filtration. *J. AWWA*, 78:5:66-73, 1986.

17. Logsdon, G.S., Thurman, V.C., Frindt, E.S., and Stoecker, J.G. Evaluating sedimentation and various filter media for removal of *Giardia* cysts. *J. AWWA*, 77:2:61-66, 1985.

18. Robeck, G.G., Clarke, N.A., and Dostal, K.A. Effectiveness of water treatment process in virus removal. *J. AWWA*, 54:10:1275-1290, 1962.

19. McCormick, R.F. and King, P.H. Factors that affect use of direct filtration in treating surface waters. *J. AWWA*, 74:5:234-242, 1982.

Principles of Drinking Water Disinfection for Pathogen Control

by: John C. Hoff
Risk Reduction Engineering Laboratory
Drinking Water Research Division
U.S. Environmental Protection Agency
26 W. Martin Luther King Dr.
Cincinnati, Ohio 45268
(513) 569-7331

Introduction

The 1986 Amendments to the Safe Drinking Water Act mandate that EPA establish disinfection requirements to ensure the control of waterborne pathogens by drinking water treatment. The requirements being developed constitute a major change in disinfection practice in the United States and are the result of application of knowledge gained over a period of more than 75 years. The purpose of this paper is to provide some insight into the basis for these changes and the principles of sound disinfection practices.

Historical Aspects

The introduction of water filtration and use of chlorine for disinfection around the turn of the century resulted in dramatic decreases in waterborne disease in the United States (1,2). Continuous application of chlorine for drinking water disinfection was first used in the United States in 1908 (3). In the same year, Chick noted the analogy between rates of inactivation of bacteria by chlorine and chemical reaction kinetics, which she incorporated into an equation now known as Chick's Law (4). Watson analyzed Chick's data in terms of the relationship of disinfectant concentration (C) and contact time (t) to killing efficiency, developing an equation now known as Watson's Law (5), which in simplified form constitutes the C·t concept (6). The C·t concept provides a major basis for the disinfection regulations currently being developed.

Subsequent developments in knowledge of chlorine chemistry led to the use of combined chlorine rather than free chlorine because of the stability of the chlorine residual. Studies on comparative biocidal efficiency, however, led to a reemphasis on free chlorine use because of its greater killing ability. This was reinforced by the results of studies in the 1950s on inactivation of enteric viruses, which were shown to be extremely resistant to chloramines. The recognition of giardiasis as a major cause of waterborne disease outbreaks in the 1970s provided additional emphasis on the need for more stringent disinfection requirements, because of its resistance to chlorine at the then normally applied dosage and contact times. Concurrently, the recognition of possible long-term health effects from chlorine by-products led to active consideration of other chemical disinfectants as alternatives to chlorine.

Relationships of Pathogen Characteristics to Disinfection Efficiency

The pathogens that must be controlled by drinking water disinfection (bacteria, viruses, protozoans) comprise a diverse group with regard to occurrence, size, mode of existence, and resistance to drinking water disinfectants. The primary source of almost all of the important drinking waterborne pathogens is the intestinal tract of animals. The notable exception is *Legionella pneumophila*, a respiratory pathogen that multiplies, apparently in association with amoeba, in warm water environments, including drinking water distribution systems. Because of its life cycle, this pathogen must be controlled by means other than disinfection during water treatment. With the exception of *Legionella pneumophila*, there is little or no evidence that any of the important drinking water pathogens multiply significantly in the water environment outside an animal host. Other characteristics that relate to control of these pathogens by drinking water disinfection include host specificity, infectious dose, innate disinfectant resistance, and size and its relationship to the effects of turbidity on disinfection efficiency.

Host Specificity

Bacterial enteric pathogens normally multiply in the intestinal tract of humans and other animals. Some show a degree of host specificity but many can infect a broad range of hosts (e.g., *Salmonella*, *Campylobacter*). In contrast, the enteric virus pathogens show a high degree of host specificity and there is no evidence of waterborne outbreaks caused by viruses from other than human sources. Consequently, water systems using source waters from protected watersheds not subjected to wastes from human sources are not required to orient their disinfection processes toward control of viruses. This becomes an important consideration when certain disinfectants, notably chloramines, are used because of the extremely high resistance of viruses to this disinfectant.

With regard to waterborne protozoan pathogens, host specificity is not yet clear. For *Giardia lamblia*, the waterborne protozoan pathogen currently of most concern, problems of lack of species definition, apparent strain-specific cross transmission characteristics, and technical problems with many of the cross-transmission studies, make it difficult to assess host specificity (7,8). Several waterborne outbreaks do appear to have resulted from source water contamination by animals other than humans (9). For *Cryptosporidium*, a protozoan pathogen that is currently gaining significance as a recognized waterborne pathogen, cross-species transmission seems to occur readily (10).

Infectious dose

The infectious dose for most enteric bacterial pathogens tested is very high (10^4 to 10^7 organisms), but for at least one *Shigella* strain, the ID_{50} was shown to be much lower (<100 organisms)(11). The infectious dose for enteric viruses also seems to vary. ID_{50}s for poliovirus fed to humans varied from <10 plaque forming units (PFU) to $>1 \times 10^4$ PFU in a number of published studies (11,12) depending on host age, virus isolate used, and other factors. Infectious dose data for viruses implicated as etiologic agents in waterborne disease outbreaks (Norwalk, Hepatitis A, rotavirus, etc.) are not available.

Similarly, for *G. lamblia*, results of infectious dose studies are not consistent. Studies by Rendtorff (13) and Hibler *et al.* (14) indicate ID_{50}s of <10 cysts in most cases, while Visvesvara *et al.* (15) reported ID_{50}s of 100 to 1,000 cysts and higher. Host specificity probably influenced the results of these studies.

Overall, it would appear that the infectious dose for waterborne pathogens, particularly viral agents and *Giardia* cysts, can be very low and therefore removal and/or inactivation of these pathogens must be conducted so as to be highly effective.

Innate resistance to disinfectants

Different types of waterborne pathogens show an overall similar pattern of relative resistance to chlorine, the most commonly used disinfectant, and to the alternative disinfectants currently considered as viable alternatives (chlorine dioxide, ozone, and chloramine). The degree of difference in resistance and, in some cases, the order of resistance vary. In general, the bacterial pathogens are the most sensitive overall, with the viral agents more resistant and the protozoans much more resistant than either of the other two groups (Table 4.3.1). Note that *E. coli*, representing enteric bacterial pathogens, is as sensitive as or, in most cases, more sensitive than the other agents. The viral agents show differences in their resistance to specific disinfectants and are extremely resistant to chloramine. *Giardia* cysts overall are more resistant to all disinfectants except chloramine. Chlorine dioxide and ozone are much more effective for cyst inactivation than chlorine or chloramine.

Table 4.3.1. Summary of C·t Value Ranges for 99 Percent Inactivation of Various Microorganisms by Disinfectants at 5°C

	Disinfectant			
Microorganism	Free Chlorine pH 6 to 7	Preformed Chloramine pH 8 to 9	Chlorine Dioxide pH 6 to 7	Ozone pH 6 to 7
E. coli	0.034-0.05	95-180	0.4-0.75	0.02
Polio 1	1.1-2.5	768-3,740	0.2-6.7	0.1-0.2
Rotavirus	0.01-0.05	3,806-6,476	0.2-2.1	0.006-0.06
Phage f_2	0.08-0.18	–	–	–
G. lamblia cysts	47 - > 150	–	–	0.5-0.6
G. muris	30-630	1,400	7.2-18.5	1.8-2.0

Adapted from Hoff (6).

Because of their high degree of resistance, *Giardia* cysts are the "target pathogen" in most cases in applying C·t values, as proposed under the regulations being developed (16). In some instances, however, viral pathogens are the target. More detailed discussions of the specific application of C·t values for the proposed regulation are given elsewhere in this volume (17, 18).

Size

The different pathogens vary in size by more than two orders of magnitude. The viral agents range from 0.02 to 0.07 m in diameter, the enteric bacteria are on the order of 0.3 by 1 m, and *Giardia* cysts are about 10 to 15 m in diameter. These differences have important consequences with regard to their removal by some filtration processes (19) and also influence their relationships to other particulate matter including adsorption phenomena and inclusion within larger particles. These associations have important implications for disinfection effectiveness because of the possible prevention of contact between the disinfectant and the microorganism. Such protective effects have been documented by a number of investigations (20, 21, 22, 23). The high degree of protection provided coliforms in wastewater effluent solids provides indirect evidence that viruses would be similarly protected because they are much smaller than bacteria. No direct evidence of *Giardia* cyst protection by this mechanism is yet available. Because they are about 10-fold larger than bacteria, this type of protection may be of less significance for *Giardia* cysts.

Such protection underscores the need for removal of potentially protective particles prior to disinfection. C·t values have been developed using purified preparations of microorganisms and this should be kept in mind in applications to real-life situations. The protective effects of organic particulate matter would render these C·t values inadequate for effective disinfection unless the particulate matter was removed.

Water characteristics

In addition to the factors described above relating to the types and sources of pathogens present in the water, two other factors, water temperature and pH, have important effects on disinfection efficiency. Temperature effects are predictable, with inactivation rates increasing at higher water temperatures. The available data indicate that inactivation rates increase two- to threefold for every 10°C increase in temperature (6).

The effects of pH are disinfectant specific. In general, within the pH range encountered in drinking water treatment, both free chlorine and chloramine become less effective as pH increases, chlorine dioxide becomes more effective as pH increases, and ozone effectiveness is relatively unchanged with changes in pH.

Little can be done to increase water temperature above ambient conditions to enhance disinfection efficiency but water temperature must be considered in evaluating disinfection requirements. While water pH can be easily changed, corrosion control rather than enhanced disinfection efficiency has usually been the primary consideration in making such changes. Corrosion control generally results in higher pH values and has often worked to the detriment of disinfection efficiency, when chlorine or chloramine is used for disinfection.

Mixing

Obviously, thorough mixing of the disinfectant with the water being treated is necessary for effective disinfection. Although this is widely recognized, the need for effective mixing does not seem to receive the attention it deserves. For instance in the AWWA handbook (1), effective mixing is discussed only as it relates to coagulation and sedimentation, not as it relates to disinfection.

Summary

The pathogens that must be inactivated by drinking water disinfection comprise a diverse group of microorganisms (bacteria, viruses, protozoans) with regard to occurrence, size, mode of existence, and resistance to disinfectants. Of the many chemical and physical disinfecting agents available, only a few have been widely applied for large-scale drinking water treatment in the United States or other countries. These include free and combined chlorine, chlorine dioxide, and ozone. The kinetic nature of microorganism inactivation by disinfectants was described many years ago and much information has been developed on the comparative resistance of microorganisms and the comparative effectiveness of disinfectants. Only recently have efforts been made to apply this information to the development of scientifically based drinking water disinfection requirements. This approach is based on use of disinfectant concentrations (C) and contact time (t) required for the inactivation of target pathogens under various conditions of water pH and temperature. While the effects of temperature increases are consistent with all disinfectants and pathogens, i.e., inactivation rates increase as temperature increases, pH effects vary depending on the disinfectant and target pathogen.

References

1. American Water Works Association. Water Quality and Treatment, McGraw- Hill, New York, New York, 1971.
2. Craun, G.F. Surface water supplies and health. *J. AWWA*, 80(2):40-52, 1988.
3. Craun, G.F. Statistics of waterborne outbreaks in the U.S. (1920-1980). *In*: Waterborne Diseases in the United States. CRC Press, Inc., Boca Raton, Florida, pp. 3-10. 1986.
4. Chick, H. An investigation of the laws of disinfection. *J. Hyg.* 8:92- 158, 1908.
5. Watson, H.E. A note on the variation of the rate of disinfection with change in the concentration of disinfectant. *J. Hyg.* 8:536-542, 1908.
6. Hoff, J.C. Inactivation of microbial agents by chemical disinfectants. EPA/600/2-86/067 U.S. Environmental Protection Agency, Cincinnati, Ohio, 1986.
7. Woo, P.K. Evidence for animal reservoirs and transmission of *Giardia* infection between animal species. *In*: Erlandsen, S.L. and E.A. Meyer (eds.), Giardia and Giardiasis. Plenum Press, New York, New York, pp. 379-400, 1984.
8. Bemrick, W.J. Some perspectives on the transmission of giardiasis. *In*: Erlandsen, S.L., and E.A. Meyer (eds.) Giardia and Giardiasis, Plenum Press, New York, New York, pp. 379-400, 1984.
9. Craun, G.F. and W. Jakubowski. Studies of waterborne giardiasis outbreaks and monitoring methods. *In*: Proc. Intnl. Symp. on Water- Related Health Issues. Am. Water Resource Assoc., Bethesda, Maryland, pp. 167-174, 1986.
10. Crawford, F.G. and S.H. Vermund. Human cryptosporidiosis. *CRC Critical Rev. Microbiol.* 16:113-159, 1988.
11. National Academy of Sciences. Microbiology of drinking water. *In*: Drinking Water and Health, Vol. 1. National Academy of Sciences, Washington, D.C., pp. 63-134, 1977.
12. Ward, R.L. and E.W. Akin. Minimum infective dose of animal viruses. *CRC Critical Rev. Env. Control.* 14:297-310, 1984.
13. Rendtorff, R.C. The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. *Am. J. Hyg.* 59:209-220, 1954.
14. Hibler, C.P., C.M. Hancock, L.M. Perger, J.G. Wegrzyn, and K.D. Swabby. Inactivation of *Giardia* cysts with chlorine at 0.5°C. *Water Treatment and Operations.* American Water Works

Association Research Foundation, Denver, Colorado, 1987.

15. Visvesvara, G.S., J.W. Dickerson, and G.R. Healy. Variable infectivity of *Giardia lamblia* cysts for Mongolian gerbils (*Meriones unguiculatus*). *J. Clin. Microbiol.* 26:837-841, 1988.

16. U.S. EPA. National primary drinking water regulations. *Fed. Reg.* 52(212):42178-41222, 1987.

17. Clark, R.L. Determination of C·T values. *In:* G.F. Craun (ed.) Methods for the Investigation and Prevention of Waterborne Disease Outbreaks. Report No. EPA/600/1-90/005a, U.S. Environmental Protection Agency, Cincinnati OH, 1990. pp. 193-206.

18. Berger, P.S. and Regli, S. The Safe Drinking Water Act and the regulation of microorganisms in drinking water. *In:* G.F. Craun (ed.) Methods for the Investigation and Prevention of Waterborne Disease Outbreaks. Report No. EPA/600/1-90/005a, U.S. Environmental Protection Agency, Cincinnati OH, 1990. pp. 23-28.

19. Logsdon, G.S. Principles of water filtration. *In:* G.F. Craun (ed.) Methods for the Investigation and Prevention of Waterborne Disease Outbreaks. Report No. EPA/600/1-90/005a, U.S. Environmental Protection Agency, Cincinnati OH, 1990. pp. 169-183.

20. Hoff, J.C. The relationship of turbidity to disinfection of potable water. *In*: C.W. Hendricks (ed.) Evaluation of the Microbiology Standards for Drinking Water. Report No. EPA-570/9-78-00C, U.S. Environmental Protection Agency, Washington, D.C., 1978. pp. 103-117.

21. LeChevallier, M.W., T.M. Evans, and R.J. Seidler. Effects of turbidity on chlorination efficiency and bacterial persistence in drinking water. *Appl. Env. Microbiol.* 42:159-167, 1981.

22. Hejkal, T.W., F.M. Wellings, P.A. LaRock and A.L. Lewis. Survival of poliovirus within organic solids during chlorination. *Appl. Environ. Microbiol.* 38:114-118, 1979.

23. Berman, D., E.W. Rice and J.C. Hoff. Inactivation of particle-associated coliforms by chlorine and monochloramine. *Appl. Environ. Microbiol.* 54:507-512, 1988.

Determination of C·t Values

by: Robert M. Clark, Director
Drinking Water Research Division
Risk Reduction Engineering Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268
(513) 569-7201

Introduction

Amendments to the Safe Drinking Water Act (PL93-523) highlight the continuing problem of waterborne disease and mandate EPA to promulgate (a) criteria by which filtration will be required for surface water supplies and (b) disinfection requirements for all water supplies in the United States. EPA's Office of Drinking Water is proposing to use the C·t (Concentration times Time – disinfectant residual concentration in mg/L multiplied by the disinfectant concentration contact time in minutes) concept for determining the inactivation of *Giardia lamblia*, one of the most resistant pathogens, likely to be present in surface waters.

Among the known causes of waterborne outbreaks, *Giardia* is the most frequently identified etiologic agent and is responsible for more cases of waterborne illness than any other agent (1). The Office of Drinking Water is developing criteria under which utilities using surface water would be required to meet source water quality conditions, maintain a protected watershed, and achieve C·t values which provide a 99.9 percent inactivation of *Giardia lamblia* cysts, in order to avoid filtration. If, for example, a utility in addition to meeting other requirements, can demonstrate that through effective disinfection, manifested by a sufficient C·t value, it can reduce *Giardia* levels by 99.9 percent, then it would not be required to filter.

In this chapter a mathematical model is developed based on the C·t concept, for inactivation of *G. lamblia* cysts by free chlorine. The model is first applied to inactivation data from animal infectivity studies. A procedure is then developed to select the best combination of data sets available assuming that the animal infectivity data are included in each combination. The model is then applied to the combined data set to calculate the model parameters. A regulatory strategy is proposed for applying this model for the determination of C·t values under the surface water treatment rule (SWTR).

The C·t Concept

The C·t concept in current use is an empirical equation stemming from the early work of Watson and is expressed as (2):

$$K = C^n t \qquad (1)$$

where

K = constant for a specific microorganism exposed under specific conditions

C = disinfectant concentration

n = constant, also called the "coefficient of dilution"

t = the contact time required for a fixed percent inactivation

It is based on the van't Hoff equation used for determining the nature of chemical reactions in which the value n determines the order of the chemical reaction (3,4).

The application of this equation to disinfection studies requires multiple experiments where the effectiveness of several variables, such as pH, temperatures, and the disinfectant concentration are examined to determine how they affect the inactivation of microbial pathogens. The disinfection concentration (C) and time (t) necessary to attain a specific degree of inactivation (e.g., 99 percent) are plotted on double logarithmic paper. Such a plot results in a straight line with slopes (5,6). Figure 4.4.1 illustrates data plotted in this manner and also the significance of the value of n in extrapolation of disinfection data (7). When n equals 1, the C·t value remains constant regardless of the disinfectant concentration used, i.e., disinfectant concentration and exposure time are of equal importance in determining the inactivation rate, or the C·t product, K. If n is greater than 1, disinfectant concentration is the dominant factor in determining the inactivation rate while if n is less than 1, exposure time is more important than disinfectant concentration. Thus, the value of n is very important in determining the degree to which extrapolation of data from disinfection experiments is valid. In addition, Morris pointed out that the evaluation of n is valid only if the original experimental data follow Chick's Law (i.e., the rate of organism distraction is directly proportional to the number of living organisms remaining at any specified time), which is normally not the case (8).

Factors Affecting C·t

The destruction of pathogens by chlorination is dependent on a number of factors, including water temperature, pH, disinfectant contact time, degree of mixing, presence of interfering substances (which may be related to turbidity), and concentrations of chlorine

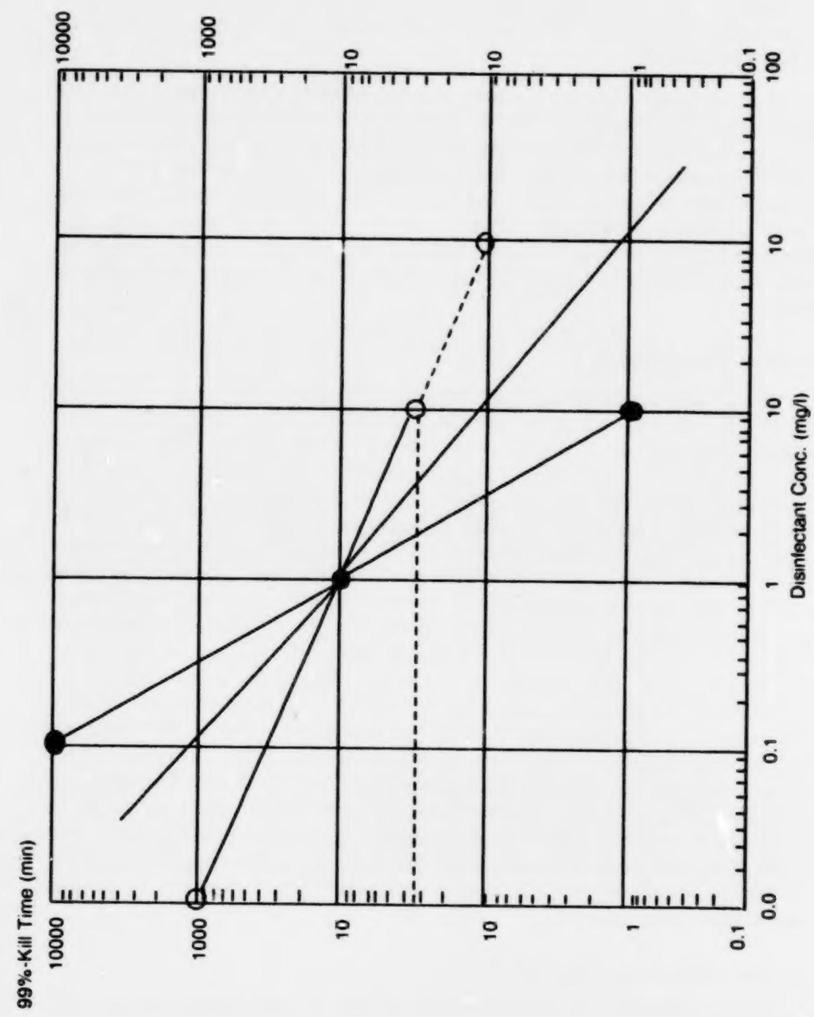


Figure 4.4.1. Effect of n value on C·t values at different disinfectant concentrations (C·t values given in parentheses).

available (7). The pH, especially, has a significant effect on inactivation efficiency because it determines the species of chlorine found in solution.

The impact of temperature on disinfection efficiency is also significant. For example, Clarke determined that in order to maintain the same level of virus destruction by chlorine, contact time must be increased two to three times when the temperature is lowered 10°C (9). Disinfection by chlorination can inactivate *Giardia* cysts, but only under rigorous conditions. Most recently, Hoff *et al.* concluded that these cysts are among the most resistant pathogens known, and that inactivation by disinfection at low temperatures is especially difficult (10).

In Vitro Excystation

Excystation, the process by which the trophozoite breaks out of the cyst wall, is considered an acceptable criterion for determining the effects of chlorination on the viability of *Giardia* cysts (10). Using *in vitro* excystation, Jarroll *et al.* have shown that 99.8 percent of *G. lamblia* cysts can be killed by exposure to 2.5 mg/L of chlorine for 10 minutes at 15°C at pH 6, or after 60 minutes at pH 7 or 8 (11). At 5°C, exposure to 2 mg/L of chlorine killed 99.8 percent of all cysts at pH 6 and 7 after 60 minutes (11). While it required 8 mg/L to kill the same percentage of cysts at pH 6 and 7 after 10 minutes, it required 8 mg/L to inactivate cysts to the same level at pH 8 after 30 minutes. Inactivation rates decreased at lower temperatures and at higher pHs, as indicated by the higher C·t values. Figures 4.4.2 and 4.4.3 summarize the data developed by Jarroll *et al.* It should be noted that the nature of the excystation method limits the ability to measure percent survival at high inactivation levels. The assay involves microscopic observation of the cysts. Therefore, to detect 1 viable cyst in 1,000 (99.9 percent inactivation), several thousand cysts must be observed to count enough viable cysts for statistical confidence at this level. Since this has not been done, no data for achieving 99.9 percent or higher inactivation levels are available from studies involving excystation procedures.

Animal Infectivity Data

Quantification of the combined effects of pH, temperature, and disinfectant concentrations require special techniques that take into account the interaction of these variables so they can be described by a single value. In this section, cyst inactivation data from animal infectivity studies conducted by Hibler *et al.* are described (12). The Hibler data are unique in that, unlike data from excystation studies, they indicate the disinfectant conditions necessary to achieve greater than 99.9 percent inactivation of *G. lamblia* cysts. These data could be combined with excystation data for *G. lamblia* by Rice *et al.* (13), Jarroll *et al.* (11), and Rubin *et al.* (14) to show the combined effects of

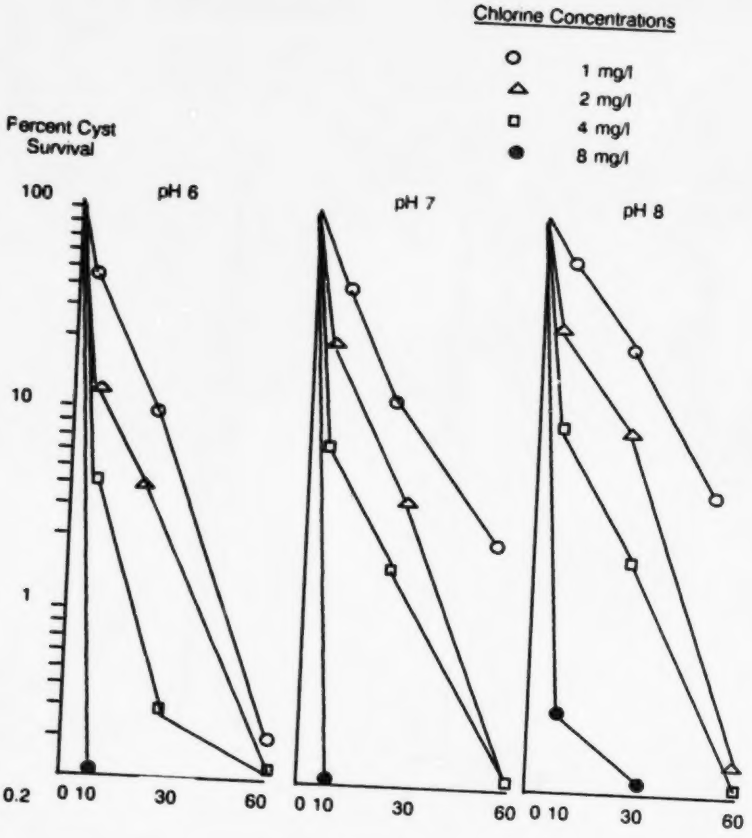


Figure 4.4.2. Inactivation of *G. lamblia* cysts by free residual chlorine at 5°C.

chlorine concentration, pH, and temperature on different levels of inactivation of *G. lamblia* cysts.

Hibler acquired *G. lamblia* isolates from several human sources and maintained them by passage in Mongolian gerbils (12). Cysts obtained from these animals were used to develop C·t values for 99.99 percent inactivation of *G. lamblia* cysts with chlorine at temperatures of 0.5, 2.5, and 5.0°C and at pH values of 6, 7 and 8.

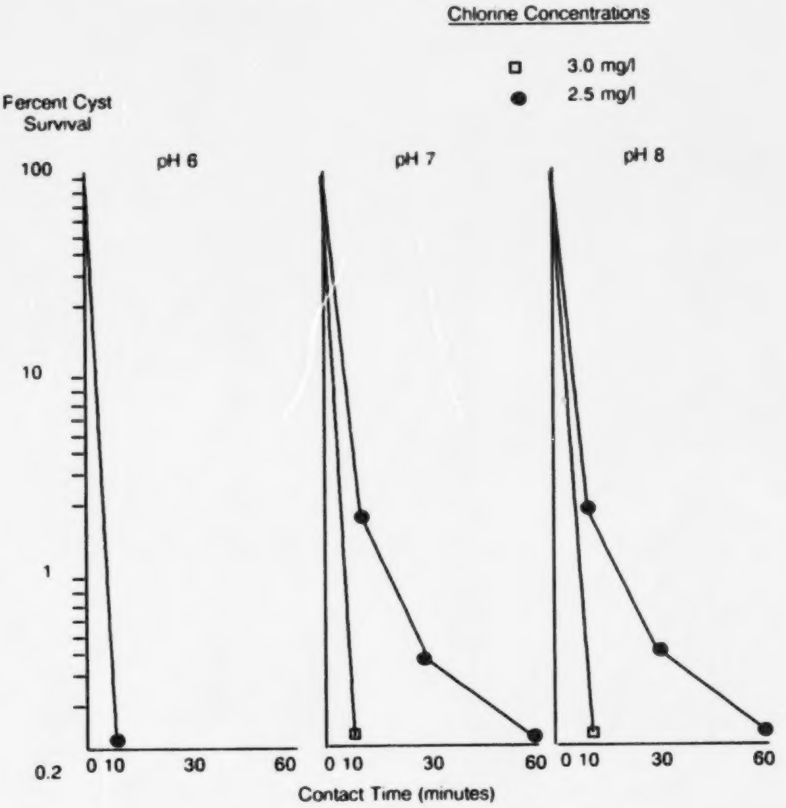


Figure 4.4.3. Inactivation of *G. lamblia* cysts by free residual chlorine at 15°C.

In these experiments, clean *G. lamblia* cysts at a concentration of 1.02 x 103 cysts/mL were exposed to selected chlorine concentrations at appropriate pH and temperature. At specified time intervals for each temperature and pH condition, chlorine activity was stopped by the addition of sodium thiosulfate. The treated cyst suspension was centrifuged, the supernatant poured off, and the cysts resuspended in a small volume of buffer. Each of five gerbils, per test run, was fed 5 x 104 of the concentrated chlorine exposed cysts. Infectivity studies with unchlorinated cysts showed that approximately five cysts usually constituted an infective dose. Table 4.4.1 shows a distribution of the number of animals infected by chlorine exposed cysts.

Table 4.4.1. Distribution of animals infected by chlorine exposed cysts.

		Number of Infected Animals					
pH	Temp	0	1	2	3	4	5
6	0.5	58	15	5	3	2	5
6	2.5	54	7	4	3	1	7
6	5	23	10	6	5	5	15
7	0.5	35	7	5	1	1	25
7	2.5	62	6	4	4	0	4
7	5	61	7	4	4	0	12
8	0.5	36	10	8	3	1	2
8	2.5	68	12	4	3	2	6
8	5	45	9	3	2	1	6

In order to analyze these data the following assumptions were made. If all five animals were infected, then the C·t of the test run produced less than 99.99 percent inactivation since cyst exposure levels would have been reduced from 5 x 10^4 to less than 5 per animal. If no animals were infected, then the C·t had produced greater than 99.99 percent inactivation, since cyst exposure levels would have been reduced from 5 x 10^4 to less than 5 per animal. In the test runs where one to four animals were infected, cyst exposure levels would theoretically have been reduced from 5 x 10^4 to both 5 or greater cysts (those infected) and to less than 5 cysts (those not infected) per animal. Since in most of the test runs where one to four animals were infected the largest number of infected animals was only one, it was considered reasonable to assume all data where one to four animals were infected represented 99.99 percent inactivation. The C·t of the test run produced less than 99.99 percent inactivation.

The limitation of this experiment is that it is only appropriate to assign a specific level of inactivation (i.e., 99.99 percent) to the case of one to four animals infected.

Model Development

Statistical analysis was performed on the infectivity and excystation data sets to determine the effects of inactivation level, temperature, pH, and concentration of disinfectant on time to inactivation (15). A multiplicative model was selected to best represent the chemical reactions during the inactivation process:

$$t = R\,I^{a}\,C^{b}\,pH^{c}\,temp^{d} \tag{2}$$

where

t = time to inactivation in minutes
I = inactivation level
C = concentration of disinfectant in mg/L
pH = pH at which experiment was conducted
temp = water temperature at which experiment was conducted
R, a, b, c, d = model parameters

A log transformation of equation 2 yields: $LOG_{10}(t) = LOG_{10}(R) + (a)LOG_{10}(I) + bLOG_{10}(C) + cLOG_{10}(pH) + dLOG_{10}(temp)$ (3)

where LOG_{10} is logarithm to the base 10.

Equation 2 was applied to each of the three data sets individually and in various combinations (Hibler *et al.* (12), Jarroll *et al.* (11), Rice *et al.* (13), and Rubin *et al.* (14). The Hibler *et al.* data were assumed to be one element in all of the combinations studied (12).

In order to arrive at the optimal combination of these data sets, equation 2 was modified to provide an alternative equation designed to study the various combinations of data as follows:

$$t = R\,I^{a}\,C^{b}\,pH^{c}\,temp^{d}\,Z^{e} \tag{4}$$

where

Z = indicator random variable
e = exponent of Z

The indicator random variable was used to examine data set compatibility and to move the regression intercept of slope to compensate for data set difference. Significance of the indicator random variable (Z) would support the hypothesis of different regression surfaces, i.e., incompatibility of the data sets chosen. The indicator random variable was created in such a way as to always differentiate between the Hibler data and other data sets considered (11,12,13,14). Table 4.4.2 contains the data set combinations and regression diagnostics.

As shown by Table 4.4.2, the indicator random variable combining the Hibler *et al.* and Jarroll *et al.* data bases was not significant. All other data bases considered had a significant indicator random variable at the 0.05 level of significance. A formal test for differences of intercept and/or slope between the Hibler *et al.* and Jarroll *et al.* data sets was conducted using the BMDP PIR procedure in the BMDP statistical programming language. Test results indicated no difference between the data sets. Thus, statistical analysis supports the choice of the Hibler *et al.* and Jarroll *et al.* data as the data base for *Giardia* cyst modeling procedures.

Table 4.4.2. Regression Diagnostics for Data Set Combinations

Data Sets Considered	R-Square	Variables	Plots
Hibler, Rice, Jarroll, Rubin	0.6801	intercept, temp not significant	non normal data non constant var
Hibler, Rice, Jarroll, Rubin, Z	0.7316	intercept, temp not significant	non normal data non constant var
Hibler, Rice, Rubin	0.6649	intercept, temp not significant	non normal data non constant var
Hibler, Rice, Rubin, Z	0.7899	intercept not significant	non normal data non constant var
Hibler, Jarroll, Rubin	0.6424	intercept, temp not significant	non normal data non constant var
Hibler, Jarroll, Rubin, Z	0.6879	intercept, temp not significant	non normal data non constant var
Hibler, Rice, Jarroll	0.8619	all variables significant	non normal data non constant var
Hibler, Rice, Jarroll, Z	0.865	all variables significant	non normal data non constant var
Hibler, Rubin	0.6483	temp not significant	non normal data non constant var
Hibler, Z	0.7593	intercept not significant	non normal data constant var
Hibler, Rice	0.8548	all variables significant	non normal data constant var
Hibler, Rice, Z	0.8678	all variables significant	non normal data constant var
Hibler, Jarroll	0.8452	all variables significant	non normal data constant var
Hibler, Jarroll, Z	0.8459	Z not significant	non normal data constant var

The resulting regression equation is:

$$t = 0.12\,I^{-0.27}\,C^{-0.81}\,pH^{2.54}\,temp^{-0.15} \qquad (5)$$

Equation 5 multiplied by C yields

$$C{\cdot}t = 0.12\,I^{-0.27}\,C^{0.19}\,pH^{2.54}\,temp^{-0.15} \qquad (6)$$

which is the C·t equation utilized. It can be shown that equation 6 is equivalent to the Watson equation (Appendix A). The confidence intervals for parameter estimation of equation 6 are:

R: (0.0384, 0.4096)
a: (-0.2321, -0.3031)
1 + b: (0.0792, 0.2977)
c: (1.9756, 3.1117)
d: (-0.0724, -0.2192)

These confidence intervals were calculated using the Bonferroni method (16).

Regulatory Application

There are many uncertainties regarding the various data sets that might be utilized for calculating C·t values. The random variable analysis shows the statistical incompatibility among these data sets. More work needs to be done to define the impact of strain variation, and *in vivo* versus *in vitro* techniques on C·t values. To provide conservative estimates for C·t values, the authors suggest the approach illustrated in Figure 4.4.4.

In Figure 4.4.4, the 99 percent confidence interval at the 4 log inactivation level is calculated. First order kinetics are then assumed so that the inactivation "line" goes through 1 at C·t = 0 and a C·t value equal to the upper 99 percent confidence interval at 4 logs of inactivation (Appendix B). As demonstrated, the inactivation line bounds higher C·t values than all of the mean C·t values from equation 6 as well as all of the Jarroll *et al.* data points (at inactivation levels of 0.1 and 0.015) and the Hibler *et al.* data points (at an inactivation level of 0.0001). Conservative C·t values, for a specified level of inactivation, can be obtained from the inactivation line prescribed by the disinfection conditions. For the example indicated in Figure 4.4.4, the appropriate C·t for achieving 99.9 percent inactivation would be 160. This approach (assumption of first order kinetics) also provides a conservative basis for establishing credits for a sequential disinfection step, e.g., the C·t values for 90 or 99 percent inactivation.

Summary and Conclusions

Amendments to the Safe Drinking Water Act clearly require all surface water suppliers in the United States to filter and/or disinfect to protect the health of their customers. *G. lamblia* has been identified as one of the leading causes of waterborne disease outbreaks in the United States. *G. lamblia* cysts are also one of the most resistant organisms to disinfection by free chlorine. EPA's Office of Drinking Water has adopted the C·t concept to quantify the inactivation of *G. lamblia* cysts by disinfection. If a utility can assure that a large enough C·t can be maintained to ensure adequate disinfection, then,

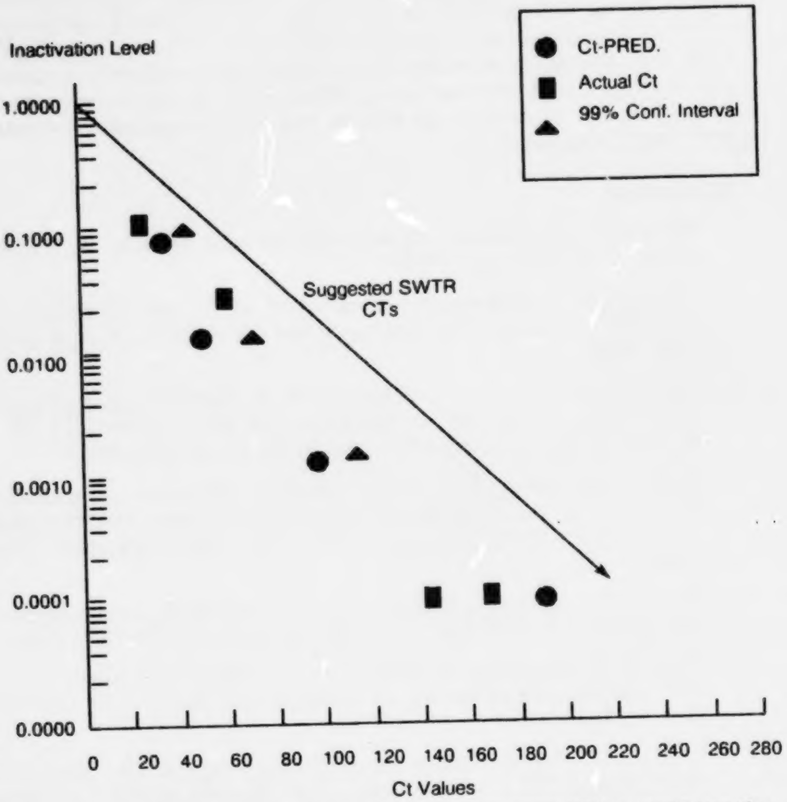


Figure 4.4.4. **Ninety-nine percent confidence levels using Hibler-Jarroll equation for chlorine = 2 mg/l; pH = 7; temperature = 5°C.**

depending upon site specific factors, installation of filtration may not be required. Similarly, the C·t concept can be applied to filtered systems for determining appropriate levels of protection.

The equation developed in this chapter can be used to predict C·t values for inactivation of *G. lamblia* by free chlorine based on the interaction of disinfectant concentration, temperature, and inactivation level. The parameters for this equation have been derived from a set of animal infectivity data (Hibler *et al.*) (12) and excystation data (Jarroll *et al.*) (11). The equation can be used to predict C·t values for achieving 0.5 to 4 logs of inactivation and, within temperature ranges of 0.5-5°C, chlorine concentration ranges up to 4 mg/L. While the model was not based on pH values above 8, the model is still considered applicable to pH levels of 9 for reasons discussed elsewhere (17). The equation shows the effect of disproportionate increases of C·t versus inactivation levels. Using 99 percent confidence intervals at the 4 log inactivation levels and applying first order kinetics to these endpoints, a conservative regulatory strategy for defining C·t at various levels of inactivation has been proposed. This approach represents an alternative to the regulatory strategy previously proposed (17).

References

1. Craun, G. F. Surface water supplies and health. *J. AWWA*. 80(2):40-56. February 1988.
2. Watson, H. E. A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. *J. Hyg*. 8:536-592, 1908.
3. Berg, G., Chang, S. L., and Harris, E. K. Devitalization of microorganisms by iodine 1. Dynamics of the devitalization of enteroviruses by elemental iodine. *Virol*. 22:469-481, 1964.
4. Fair, G. M., Geyer, J. C. and Okun, D. A. Water and Wastewater Engineering. Vol. 2 Water purification and wastewater treatment and disposal. John Wiley and Sons, Inc., New York, New York, 1968.
5. Fair, G. M., Morris, J. C., and Chang, S. L. The dynamics of water chlorination. *J. New Eng. Water Works Assoc*. 61:285-301, 1947.
6. Fair, G. M., Morris, J. C., Chang, S. L., I. Weil, and Burden, R. P. The behavior of chlorine as a water disinfectant. *J. AWWA*. 40:1051-1061, 1948.
7. Hoff, J. C. Inactivation of microbial agents by chemical disinfectants. EPA/600/2-86-067, U.S. Environmental Protection Agency, 1986.
8. Morris, J. C. Disinfectant chemistry and biocidal activities. *In*: Proceedings of the National Speciality Conference on Disinfection, American Society of Civil Engineers, New York, New York, 1970.
9. Clark, N. A., Berg, G., Kabler, P. W. and Chang, L. L. Human enteric viruses in water: Source, survival, and removability. Presented at International Conference on Water Pollution Research, Landar, September 1962.
10. Hoff, J. C., Rice, E. W., and Schaefer, III, F. W. Disinfection and the control of waterborne giardiasis. *In*: Proceedings of the 1984 Specialty Conference, Environmental Engineering Division, ASCE, June 1984.

11. Jarroll, E. L., Bingham, A. K., and Meyer, S. A. Effect of chlorine on *Giardia lamblia* cyst viability. *Appl. Env. Microbiol.*, Vol. 41, pp. 483-487, February 1981.

12. Hibler, C. P., Hancock, C. M., Perger, L. M., Wegrzn, J. G., and Swabby, K. D. Inactivation of *Giardia* cysts with chlorine at 0.5°C to 5.0°C. American Water Works Association Research Foundation, 6666 West Quincy, Denver, Colorado.

13. Rice, E. W., Hoff, J. C., and Schaeffer, III, F. W. Inactivation of *Giardia* cysts by chlorine. *In*: Applied and Environmental Microbiology, Vol. 43, pp. 250-251, January 1982.

14. Rubin, A. Internal report of progress, through June 1, 1988. EPA Project CR812238, 1988.

15. Clark, R. M., Read, E. J., and Hoff, J. C. Inactivation of *Giardia lamblia* by chlorine: A mathematical and statistical analysis. Accepted for publication by the *J. Env. Eng.*

16. Neter, J. and Wasserman, W. Applied Linear Statistical Models. Irwin: Homewood, Illinois, 1974.

17. Regli, S. EPA disinfection regulations. *In:* Seminar Proceedings Assurance of Adequate Disinfection or C·t or not C·t, American Water Works Association Annual Meeting 1987, pp. 1-7.

Appendix A

Equation 6 can be shown to be equivalent to equation 1 by dividing equation 6 by C^b which yields:

$$C^{-b}t = RI^{a}\,pH^{c}\,temp^{d} \qquad \text{(A-1)}$$

Assuming a constant $pH = \overline{pH}$,

$temp = \overline{temp}$ and $I = \overline{I}$ yields

$$K = R\overline{I}^{a}\,\overline{pH}^{b}\,\overline{temp}^{c}\,\overline{I}^{d} \qquad \text{(A-2)}$$

therefore

$$C^{-b}t = K \qquad \text{(A-3)}$$

where

$-b = n$ in equation 1

Appendix B

A general relationship that relates C·t values at different inactivation levels is:

$$\frac{\ln(N_i/N_o)}{\ln(N_j/N_o)} = \frac{K_i}{K_j} \qquad \text{(B-1)}$$

where

N_i = the number of organism left at time t_i
N_j = the number of organisms left at time t_j.

Table 4.4.B-1 summarizes the multiplication factors to be applied, assuming first order kinetics, to convert a value of K_i to an equivalent value of K_j.

Table 4.4.B-1. Multiplication Factors to Convert C·t Values from one Inactivation Level K_i to Inactivation Level K_j

From Inactivation Level i	To Inactivation Level j	Multiplier for Ki			
		90	99	99.9	99.9
90		–	2.0[1]	3.0	4.0
99		1/2	–	1 1/2	2.0
99.9		1/3	2/3	–	1 1/3
99.9		1/4	1/2	3/4	–

[1] Multiplier for Ki, e.g., to convert 90 percent inactivation to 99 percent inactivation, multiply by a factor of 2.

Distribution Systems: Treated Water Quality Versus Coliform Noncompliance Problems

by: Donald J. Reasoner, Research Microbiologist
Edwin E. Geldreich, Senior Research Microbiologist
Drinking Water Research Division
Risk Reduction Engineering Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268
(513) 569-7234

Introduction

The maintenance of water quality during distribution is a concern for every water utility, regardless of geographic location and population size served. Factors that adversely affect water quality, or the determination of water quality, must be considered with respect to the utility's ability to meet the requirements of the Primary Drinking Water Standards and alleviate the health risk to consumers.

Water quality is dynamic; it will change as the water progresses from one point to another in distribution. To prevent adverse change, the factors that cause degradation in water quality must be understood and appropriate steps taken to prevent degradation. Some of these factors may not be reasonably controlled, but it is imperative that their impact on water quality be assessed.

With respect to bacteriological quality, it is necessary to recognize that the distribution system is an ecosystem that responds to physical and chemical changes in the water passing through it. Our knowledge about those changes and their effects on the bacteria in the system is rudimentary. In general, control of bacterial populations in distribution systems has been accomplished primarily by increasing the disinfectant residual. However, it has become increasingly apparent over the last few years that this is not always effective; there are numerous instances where coliform bacteria have become established in the distribution system and show up intermittently, or in some cases pose a fairly persistent problem (1,2,3,4,5).

Distribution system problems caused 16 percent of waterborne outbreaks and illnesses during the period 1971-1985 (6). However, these figures represent only reported waterborne outbreaks; there may have been other similar outbreaks that went undetected and there have been many distribution problems that have not been epidemiologically investigated.

The occurrence of coliform bacteria in treated distribution system water raises a warning flag that a problem exists that needs to be addressed immediately. In the past, it was common to attribute coliform positive samples to improper sample collection or laboratory error, particularly when check samples turned out to be coliform free. In recent years, however, there has been sufficient documentation of intermittent and/or persistent coliform occurrences in treated distribution water systems to cause a change in attitude toward this problem. The emphasis now is on reevaluating what actually is occurring, and finding ways to control the bacterial levels in distribution systems.

Table 4.5.1 presents a summary of documented coliform occurrence problems in public water supplies in the United States and Canada, providing a perspective on this problem. These data were gathered from published reports and from personal communications (E.E.G) with state and U.S. EPA Regional personnel. Again, these are the known and documented cases; how many similar occurrences have gone either undetected or ignored is unknown.

There are two major concerns about the presence of coliforms in distribution systems: 1) When a coliform occurrence problem arises, does it signify an increased health risk? and 2) If the coliform bacteria have colonized the system and there is no increased health risk, of what value is the continued reliance on the coliform group of bacteria as a sanitary indicator? The documented problems in Table 1 have not been associated with increased infectious disease risk based on existing surveillance systems. However, specific epidemiologic studies to determine this risk were not conducted. On the other hand, waterborne outbreaks have resulted in instances when coliforms were found in the distribution system. How can coliform occurrences associated with unsafe water quality be effectively differentiated from those associated with safe water quality? At present, there has been no research conducted to effectively answer the latter question and we must treat all coliform occurrence problems as indicative of a potential health problem in treatment or distribution, or both.

The geographical distribution of systems with coliform problems is shown in Figure 4.5.l. As indicated, the problems have occurred primarily in the eastern half of the United States. This may be due to the age of the distribution systems involved and to the source water.

Origins of Microbial Flora in Drinking Water

The origins of the microbial flora in drinking water include source water, treatment process exposures, formation of biofilms in the distribution system, and post treatment contamination caused by events such as main breaks and repairs, and cross-connections (Table 4.5.2). Source water and treatment process contributions are due,

Table 4.5.1. Coliform Occurrences in Public Water Supply Distribution Systems

Location	Population[a]	Reference
United States:		
Nassau County, NY (Fairfax Water Authority)	2,600,000	—[b]
San Francisco, CA	678,974	Tracy et al. (7)
Milwaukee, WI (summer fair line)	964,988	Milwaukee Journal (8)
New Haven, CT	761,337	Centers for Disease Control (9)
Worchester, MA	646,352	—[b]
Fairfax, VA	598,601	The Fairfax Journal (10)
Rochester, NY	241,741	Rochester Democrat and Chronicle (11)
Akron, OH	237,177	Akron Beacon Journal (12)
Lexington, KY	204,165	—[b]
Grand Rapids, MI	181,843	Wierenga (3)
Flint, MI	159,611	—[b]
Ann Arbor, MI	107,316	—[b]
Springfield, IL	99,637	Hudson et al. (2)
Miami Beach, FL	96,298	AWWA Mainstream (13)
Muncie, IN	95,000	Earnhardt (14)
Salem-Beverly, MA	75,875	McFeters et al. (15)
Bethlehem, PA	70,419	—[b]
Monmouth, NJ	68,000	—[b]
Terre Haute, IN	61,125	—[b]
Florissant, MO	55,372	St. Louis Post-Dispatch (16)
Asheville, NC	53,281	O'Brien and Gere (17)
Wilmette, IL	28,229	—[b]
N. Andover, MA	16,284	—[b]
Bennington, VT	16,000	McFeters et al. (15)
Brockport, NY	9,776	Clark (18)
Seymour, IN	4,600	Lowther and Moser (1)
Lakewood, NY	3,941	Lakewood Post-Journal
Kennebunk, ME	3,294	McFeters et al. (15)
Newfields, NH	700	—[b]
Eden Isles, LA	500	Times-Picayune (20)
Total	8,120,652	(continued)

Table 4.5.1. (Continued)

Location	Population[a]	Reference
Canada:		
Edmonton, Alberta	461,361	—[b]
Vancouver, British Columbia	410,188	—[b]
Halifax, Nova Scotia	117,882	Martin et al. (5)
Total	989,431	

[a] Population based on 1980 census, or on population given in reference.

[b] Where references are not given, information was collected by E.E. Geldreich through personal communications with State or U.S. EPA Regional Water Supply representatives.

respectively, to survival of source water bacteria that pass through the treatment processes and bacteria from biofilm that sloughs from rapid sand and carbon filters. Turbidity particulates, including carbon fines from granular activated carbon filter beds and floc, may have bacteria associated with them that are protected from the disinfectant. Water supply treatment was not intended to produce sterile water but to remove organisms of risk to public health. Coliform bacteria were chosen as indicators of pathogen presence because of their longer survival in the water environment and their presence may indicate deficiencies in water treatment.

Water Pipe Environment

Once they pass through the treatment barriers, some of the bacteria find conditions in which they can survive, adapt, and grow, thus supporting the idea of the distribution system as an ecosystem or natural habitat.

The distribution system becomes a repository for particulates that include carbon fines, floc particles, soil particles, and biological materials such as algae, bacteria, protozoa, etc. In addition, nutrients dissolved in the water are available for bacterial growth, pipe walls and sediments provide sorption sites for bacteria and nutrients, water pH conditions are very suitable for bacterial growth, and the water temperature may be conducive to bacterial growth, particularly if it is above 10°C. Finally, flow conditions may favor bacterial growth particularly those areas of slow or no flow (dead-ends) in the distribution network. In fact, all conditions in the distribution system except the presence of a disinfectant residual may contribute to the growth of numerous heterotrophic bacteria.

Biofilms in Distribution Systems

Once in the distribution system environment, many bacteria reach the pipe walls and sediments where they find conditions suitable for survival and possibly growth. Bacteria may attach to the surfaces of

Table 4.5.2. Sources of Microorganisms in Distribution Water

Source	Microorganism(s)	Reference
Wood	*Klebsiella*	Seidler et al. (21)
Filter sand; filter effluent piping; filter bed	*C. freundii, E. coli,*	Wierenga (3)
Pipe tubercles, floc, sediment, pipe biofilm	*E. coli, K. pneumoniae K. oxytoca, E. cloacae, E. agglomerans*	LeChevallier et al. (4)
Pipe tubercles, pipe biofilm	*K. pneumoniae, K. oxytoca E. agglomerans, E. cloacae E. coli, C. freundii*	Lowther and Moser (1)
Treatment plant	*K. pneumoniae, E. cloacae Enterobacter* sp.	Hudson et al. (2)
Biofilm	*K. pneumoniae, K. ozaenae, K. rhinoscleromatis, E. coli, E. cloacae, E. aerogenes, E. agglomerans, C. freundii*	Reilly and Kippin (22)
GAC, sand, anthracite filter beds	*K. oxytoca, K. pneumoniae, E. coli, E. agglomerans, E. cloacae, E. aerogenes, E. sakazaki, C. freundii, Serratia* sp.	Camper et al. (23,24)
Invertebrates	*Aerobacter aerogenes (Enterobacter aerogenes)*	Tracy et al. (8) Levy et al. (25)
Nematodes		Chang et al. (26)
Open finished water reservoir	Total coliforms	Silverman et al. (27)
Treatment breakthrough, pipe break	*E. aerogenes, E. agglomerans, K. pneumoniae, K. oxytoca*	McFeters et al. (15)

pipes or particles by means of glycocalyx, which is composed of complex mucopolysaccharide material. The organisms may also respond to the low nutrient conditions by forming a capsule, which also provides some protection from disinfection. Once attached to the pipe or sediment surfaces, the organisms benefit by the presence of other organisms and their capsular and glycocalyx materials, and possibly by symbiotic interactions in which metabolites released by other organisms may be used as nutrients and vice versa. Biofilms develop in all aquatic ecosystems and water distribution systems are no exception (28, 29).

The interior of a pipe that has become corroded and tuberculated, or that has developed a porous coating of inorganic minerals provides tremendous surface area for bacterial colonization (30,31,32,33). It is important to emphasize that no water system is free from biofilm; what is most important is the degree of development of the biofilm. The development of biofilms that include coliform colonization is believed to require relatively high levels of assimilable organic carbon (AOC) in order for the coliforms to multiply. Conditions that favor the release of coliform-containing biofilm from the pipe or sediment surfaces contribute to the occurrence of coliforms in water samples taken for analysis. Most coliform occurrence problems that have been investigated have involved species of *Klebsiella*, *Enterobacter*, or *Citrobacter*. Occasionally, *Escherichia coli* has been found, but the infrequency of its occurrence indicates that this organism generally does not colonize and grow in the biofilm. Rather, it remains an indicator of recent sewage or fecal contamination.

Distribution system coliform problems have involved, or been compounded by, such things as improper operation of filters and inadequate or interrupted disinfection, as well as problems with system maintenance. Dead-end and slow-flow areas of distribution systems contribute highly to bacterial growth due primarily to the loss of disinfectant residual and its inhibitory effect on bacterial growth. For example, when sufficient nutrients are available in a standpipe or storage reservoir that is not used for some period of time, coliform bacteria may grow and then be dispersed when water is moved into the distribution system. A coliform problem in Florrisant, Missouri, was due to water that had been held for several months in a standby reservoir. In another case, residual food grade antifreeze used to protect a water line to a festival site from freezing during the winter non-use period provided nutrients for coliform regrowth the following summer. The problem was cleared up by continuous line-flushing in the spring until a low HPC was achieved and no coliforms were detected in 100-mL samples; booster chlorination was applied during the summer-use period to maintain a chlorine residual.

Water main repairs and new main construction may result in coliform problems, usually because of inadequate flushing and disinfection. Construction materials, such as wood, left in newly constructed lines, have been implicated in coliform problems. In Halifax, Nova Scotia, *Klebsiella pneumoniae* grew in association with wooden material left from construction (5). To resolve the problem, lime was added to adjust the pH to 9.1, which either inactivated the organisms, or resulted in the formation of a carbonate scale that trapped the organisms so that they could not slough off into the water.

The hydraulic effects of high-flow rates and high pressure can also contribute to coliform problems, shearing biofilm organisms from the pipe or sediment surfaces. High pressures can cause breaks in pipe when pressure fluctuates suddenly due to rapid changes in demand. Temporary flow reversals caused by sudden heavy demands in a portion of the system can stir up and resuspend sediments, or cause biofilms to shear or break loose.

As indicated earlier, many of the systems that have identified a coliform noncompliance problem are east of the Mississippi River and north of the Ohio River (Figure 4.5.1). Characteristics shared by water systems in this area include use of surface source waters, a significant percentage of iron pipe in the system having been in service for more than 75 years, and tuberculations in the iron pipe. Additionally, systems with a problem of bacterial regrowth or aftergrowth frequently lack an adequate disinfectant residual, or are unable to maintain an adequate residual throughout the distribution system. The microbial growth problem becomes worse during the warmer period of the year, generally summer to late summer and even late fall in many areas of the United States. Bioadsorption of nutrients during cold water periods may make more nutrients available for bacterial growth during warm water periods.

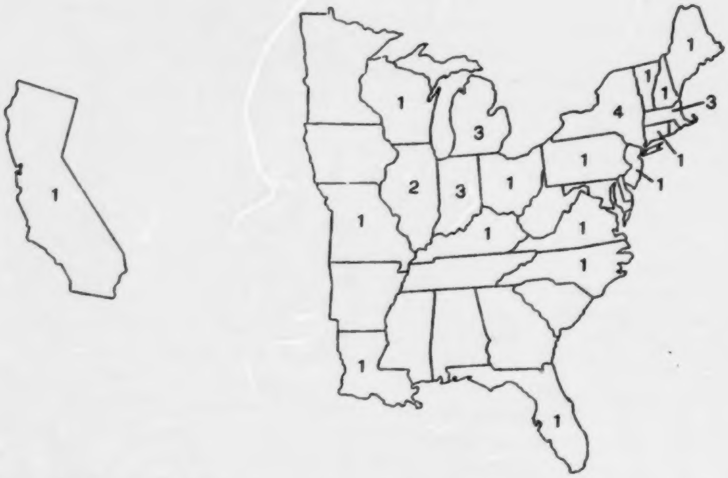


Figure 4.5.1. Distribution of U.S. public water supplies with coliform occurrences (numbers = occurrences).

The nutrient concentrations in the bulk water needed to support coliform regrowth are probably greater than 50 to 60 µg/L measured by an assimilable organic carbon bioassay (34). Nutrient levels needed for the growth of noncoliforms are probably less than this, although van der Kooij has indicated that general bacterial growth in distribution water may be limited at AOC levels below 10 to 15 µg acetate carbon equivalents/L (34). The magnitude of the actual nutrient concentrations organisms encounter at the interface between the water and the pipe or particle surfaces where they reside is currently unknown.

Coliform Case Study – Multiple Factors

Because of the difficulties many utilities have experienced in solving persistent coliform problems, it is evident that a variety of factors are involved and that simple solutions, such as boosting the chlorine residual, are either not readily available or not satisfactory. Differing circumstances at each utility have required different remedies; in all cases, multiple factors have affected both the coliform persistence and the remedy. Some utilities have not been able to eliminate the coliform problem entirely and are faced with being out of compliance with the coliform Maximum Contaminant Level (MCL) during some portions of the year, or being close to being out of compliance much of the time, both particularly during warm water months.

In a recently published study of a persistent coliform problem in New Jersey, coliforms were first isolated in June 1984 and have persisted at varying levels since that time (35). The water received conventional treatment (pretreatment, flocculation, clarification, filtration, and postdisinfection). In studying this problem, investigators concluded that the treatment plant was not the main source of coliforms since the plant met the coliform standard. However, coliforms were found in 193 of 500 (38.6 percent) of the distribution samples collected between May and August 1986. Coliform numbers increased between the treatment plant (0.03/100 mL) and the first distribution site (0.64/100 mL), and then decreased (0.20/100 mL) as the water moved through the study area. Not only did coliform numbers increase after treatment, but the species diversity also increased. Coliforms found included *E. agglomerans* (44.2 percent) at all locations, *E. cloacae* (24.4 percent) at all locations, *K. oxytoca* (predominant in past episodes), *K. pneumoniae*, *E. coli* (6 percent), and others. Noncoliform bacteria (HPC) levels often exceeded 10^6 CFU/mL at the end of branch lines and included *Flavobacterium* and *Pseudomonas vesicularis*. AOC levels in this study decreased from about 130 µg/L in the treatment plant effluent to 56 µg/L at a distribution sample site, a total decrease of 67 percent. Laboratory studies showed that *E. coli* grew almost fourfold in dechlorinated treatment plant effluent, but essentially did not grow in water from the last distribution site with an AOC of about 56 µg/L.

Coliforms were found in pipe sediments at about 0.5 CFU/g sediment and in tubercules released by the cleaning operation (pigging) at >160 CFU/g tubercule. No coliforms were found in a sample taken from a 20 cm^2 area of cement pipe before cleaning, but the sampling effort may not have been intensive enough to detect coliforms. Ridgway and Olson have shown that the occurrence of bacterial microcolonies on pipe walls is sparse and randomly distributed (patchy) (33); coliform occurrence within the biofilm is likely to be patchy also.

Coliform bacteria were present in a variety of samples from this system, sometimes at high densities such as in the tubercle material. Although the water leaving the treatment plant contained only 0.03 coliforms/100 mL (30 times lower than the standard), fluctuations in various treatment parameters such as pH and chlorine indicated the need for tighter control on some of the plant processes.

Simple calculations indicate that at a treatment effluent quality of 0.03 coliforms/100 mL and a mean production of 32.7 MGD (range of 23.3 to 43.0 MGD), the mean input into the distribution system was 2.2×10^7 coliforms per day (range of 1.5×10^7 to 2.8×10^7 coliforms per day). Thus, a continuous seeding of the distribution system was occurring. In addition, although the average free chlorine residual of the water was 1.1 mg/L (range of 0.1 to 3.0 mg/L), simple laboratory experiments with biofilm coliforms indicated only about 50 percent inactivation of coliforms after 1 hour of exposure to 1.1-mg/L free chlorine residual.

Comparison of the mean coliform level leaving the treatment plant (0.03/100 mL) with coliform levels detected in the distribution water by the standard coliform procedure using m-Endo medium (0.33/100 mL) indicated an 11-fold increase in the coliform concentration of the distribution system water. Coliform recovery by the stressed coliform procedure using m-T7 medium (0.41/100 mL) showed a 13-fold increase in coliform concentration in the distribution water; evidence that viable injured coliforms were present. Thus, not only was there a continuous seeding of the system with coliforms, but the disinfectant residual was not effective in significantly reducing the coliforms in the system. A biofilm reservoir of coliforms existed in the sediment, tubercles, and probably the pipe walls in some areas of the system.

The combination of factors in this scenario may represent situations of persistent or intermittent coliform occurrence in other distribution systems that were not as well studied and documented. A variety of such coliform problems need to be thoroughly studied and analyzed to develop better information on the critical factors involved, as well as to formulate the appropriate corrective actions to eliminate the problem. The need for action to correct problems of coliform occurrence is evidenced by the apparently increasing number of reports of intermittent and persistent coliform outbreaks in municipal water treatment and distribution systems.

Action Plan for Correction of Coliform Problem

The first task in correcting a coliform problem is determining whether the coliform occurrences represent new contamination or the recurrence of coliforms that have colonized the distribution system. New contamination indicates a penetration of the treatment barrier and may represent a risk of a waterborne outbreak. Additional coliform monitoring of the plant effluent may be instructive, including the examination of larger sample sizes, i.e., 500 mL to 1 L, or composite samples collected over a 24-hour period. Because penetration of the treatment barrier by coliforms may occur along with particulate materials, increased monitoring of the turbidity level of the treated water entering the distribution system may also be helpful. For systems that use disinfection as the only treatment barrier, storm events and increased runoff from the watershed signal a need to be more vigilant of the disinfection process and ensure the disinfectant residual is adequate to compensate for any increased chlorine demand in the water. A thorough evaluation of the treatment plant operation and correction of any deficiencies, no matter how trivial they may appear, is also needed to protect against transmission of possible pathogens and aid in determining whether the coliform problem is new contamination or possible colonization.

Other microbial determinations may yield additional useful information to evaluate health significance. HPC measurements will add data on regrowth potential. Other indicator organisms such as *Clostridium perfringens* may be useful for filtered systems; its presence indicates poorly treated contaminated water since *C. perfringens* does not grow in a pipe network.

Coliform occurrences due to treatment plant problems often appear to be widespread in the distribution system and frequently can be brought under control rapidly by correcting the treatment failure. In addition to checking the treatment plant operations, operators should thoroughly evaluate the distribution system integrity and events that impact distribution system operation. Coliform occurrences due to distribution system defects or operational problems are often characterized by positive coliform samples only in the area downstream from the defect. If due to a low pressure or reversed flow situation, the coliform occurrences may be scattered and have no specific pattern. A cross-contamination control program helps to reduce potential contamination problems due to this source. Knowledge of events that caused high water demand, such as fire fighting, can be helpful in determining the factors that may be involved in a coliform outbreak.

Regardless of whether treatment plant operation or a distribution system problem is the source of coliforms, detection of *Escherichia coli* or any other fecal coliform organism is cause for alerting the public to boil their drinking water until the problem has been rectified and coliforms can no longer be detected.

If efforts to isolate the coliform source are unsuccessful, with coliforms continuing to appear in a random fashion throughout all or parts of the distribution system, and various efforts to eliminate the coliform have failed, it is possible that the coliform colonized the biofilm on the walls of the distribution pipes. In such situations, *Klebsiella, Enterobacter*, or occasionally *Citrobacter* species predominate, even in the presence of a free chlorine residual ranging from 0.1 to 2.0 mg/L. These coliform occurrences may reflect either the existence of a biofilm growth during warm water periods or the release of biofilm as a result of an action such as a change in water pH to reduce a corrosion problem.

Each distribution system is unique and corrective measures for suppressing coliform occurrences will vary both in kind and in the degree of response. In all situations, however, an aggressive flushing program applied to all sections of the distribution system is recommended. This will clear the system of static water that may contain high densities of bacteria and colloidal particulates, which exert disinfectant demand and may reestablish a free chlorine residual if the disinfection process does not involve chloramines. There may be a period of 24 to 48 hours following the flushing when water quality temporarily deteriorates and the water may have increased coliform levels, as well as increased color, taste, and odor. Notifying the public in advance of the flushing program and asking them to run the water to waste at their taps will help restore the clean water appearance and possibly reduce the number of telephone complaints. Some areas of the distribution system may need more aggressive cleaning, such as forcing of plastic plugs through the pipe (pigging) or mechanical scraping to remove tuberculations and accumulated sediments.

In addition to flushing, it is important to establish a disinfectant residual throughout the distribution system to control the biofilm. Where free chlorine is used, it may be necessary to use 5 mg/L or more during the warm water period, and coliform levels may undergo a slow reduction rather than an immediate drop to nondetectable levels. Systems that use chloramines may find it desirable to change to an alternative disinfectant (free chlorine, chlorine dioxide) to control the heterotrophic bacterial population and better disinfectant penetration of the biofilm. In other systems, changing to the use of chloramines may result in better coliform control because of rate limiting transport of the disinfectant into the biofilm (34).

A pH adjustment may be helpful in bringing a coliform problem under control. In at least one case, adjusting the pH to 8.5 and adding lime was apparently successful in producing a less porous calcium carbonate scale, resulting in less favorable conditions for bacterial colonization. In another case, elevation of the pH to 9.1 was effective in reducing *Klebsiella* occurrence, possibly due to the destruction of polysaccharide capsular and biofilm material (5). Lowering the pH below 8.3 for corrosion control management has been implicated in several coliform problems as a factor in creating porous sediments and scale and apparently releasing embedded viable coliforms.

Since coliform colonization in the distribution system does not signify fecal contamination, the need to notify the public of coliform noncompliance requires a slightly different approach, with two action levels.

The first level of action is to form a task group to evaluate the problem and examine the laboratory results to determine if the occurrences are a result of coliform release from the biofilm in the network, and therefore of little apparent public health threat. The task group should be composed of utility, state, and federal water authorities and should jointly recommend initiation of corrective actions. It should meet at frequent intervals (weekly or daily, if necessary) to review progress by the water plant operations in correcting the deficiencies. The public should also be informed that water treatment adjustments are in progress to improve water quality. Local hospitals and medical clinics may also be alerted so they can monitor for any evidence of waterborne disease as a precautionary measure.

The second level of action is to issue a boil water order immediately if any of the following occur:

- Illness that may indicate a waterborne outbreak
- Failure to follow-up on task group recommendations
- Breakdown in disinfection treatment
- Detection of *E. coli* or fecal coliform bacteria in the water distribution system

Frequent boil water orders, however, may cause either public overreaction or apathy. One concern is that customers may seek water supply alternatives (bottled water, point-of-use treatment), some of which may be of less desirable quality than public water supplies. The overall objective is to formulate an action plan that responds to the problem and aims at correcting it, rather than driving the monthly coliform average into compliance by increased sample analyses, thus creating a false sense of compliance.

Summary

The overall quality of treated drinking water is subject to deterioration during distribution. Factors that affect the rate and degree of deterioration include source water characteristics and quality, type of treatment and treatment effectiveness, disinfectant residual, temperature, pH, flow rate, residence time, organic carbon available for microbial growth, the numbers and types of microorganisms present, and distribution system age and condition. The bacterial load of the distribution water is significantly influenced by several of these factors.

The occurrence of coliform bacteria, used as indicators of treatment effectiveness and sanitary quality, in treated distribution water causes concern because of the possibility that disease-causing organisms may also be present. Coliform occurrences may cause the utility to be out-of-compliance with the coliform MCL of the Primary Drinking Water Standards, even though the coliform organisms may pose no direct health risk. Thus, conditions that favor coliform persistence or growth within a distribution system impact our ability to discriminate between situations of real and apparent adverse health risk.

During the past few years, documentation of intermittent and/or persistent coliform problems in a number of water distribution systems, primarily in the eastern half of the United States, has stimulated interest and concern about this problem. This information suggests that there are conditions under which coliforms survive, or may actively grow, in biofilms that develop on the pipe surfaces and sediments in the distribution system. Continuing research into the underlying causes of coliform persistence/colonization in distribution systems is needed. Until our knowledge of these conditions becomes more complete, we must rely on educated guesses to formulate practical approaches to remediation and control of coliform noncompliance problems.

References

1. Lowther, E.D. and Moser, R.H. Detecting and eliminating coliform growth. *In:* Technology Conference Proceedings, WQTC-12, 1984, Advances in Water Analysis and Treatment. American Water Works Association, Denver, Colorado, 1985. pp. 323-336.
2. Hudson, L.D., Hankins, J.W., and Battaglia, M. Coliforms in a water distribution system: a remedial approach. *J. AWWA.* 75(11):564-568, 1983.
3. Wierenga, J.T. Recovery of coliforms in the presence of a free chlorine residual. *J. AWWA.* 77(11) 83-88, 1985.
4. LeChevallier, M.W., Babcock, T.M., and Lee, R.G. Examination and characterization of distribution system biofilms. *Appl. Environ. Microbiol.* 53:2714-2724, 1987.
5. Martin, R.S., Gates, W.H., Tobin, R.S., Grantham, D., Sumarah, R., Wolfe, P., and Forestall, P. Factors affecting coliform bacteria growth in distribution systems. *J. AWWA.* 74(1):34-37, 1982.
6. Craun, G. Surface water supplies and health. *J. AWWA.* 80(1) 40-52, 1988.
7. Tracy, H.W., Camarena, V.M., and Wing, F. Coliform persistence in highly chlorinated waters. *J. AWWA.* 58:1151-1159, 1966.

8. Milwaukee Journal. Summerfest: don't drink the water. Milwaukee, WI. July 3, 1985.

9. Centers for Disease Control. Detection of elevated levels of coliform bacteria in a public water supply - Connecticut. *Morbidity and Mortality Weekly Report.* 34(10):142-144. March 15, 1985.

10. Fairfax Journal, The. Consumer group critical of Alexandria Tapwater. Fairfax, Virginia. May 8, 1986.

11. Rochester Democrat and Chronicle. A puzzling bacteria invasion in water supply. Rochester, NY. April 20, 1986.

12. Akron Beacon Journal. Tainted water found in two areas. Akron, Ohio. August 10, 1983.

13. American Water Works Association. Health Department issues boil water order in Miami Beach. *AWWA Mainstream.* 27(5):1,5, 1983.

14. Earnhardt, K.B., Jr. Chlorine-resistant coliform: the Muncie, Indiana experience. *In:* Technology Conference Proceedings, WQTC-8, 1980, Advances in Laboratory Techniques for Quality Control, American Water Works Association, Denver, Colorado. pp. 371-376, 1981.

15. McFeters, G.A., Kippin, J.S., and LeChevallier, M.W. Injured coliforms in drinking water. *Appl. Environ. Microbiol.* 51:1-5, 1986.

16. St. Louis Post-Dispatch. Florissant hard-boil spurs soft drink sales. St. Louis, MO. March 6, 1984.

17. O'Brien and Gere, Consulting Engineers. Report on water quality study of Asheville, North Carolina. O'Brien and Gere, Consulting Engineers, Charlotte, NC. June, 1971.

18. Clark, T.F. Chlorine tolerant bacteria in a water distribution system. *Public Works.* pp. 65-67, 1984.

19. Lakewood Post-Journal. Expect more turbidity as water system fixed. Lakewood, NY. July 13, 1982.

20. Times-Picayune. Water supply of Eden Isles cited by state. Eden Isles, LA. October 28, 1983.

21. Seidler, R.J., Morrow, J.E., and Bagley, S.T. *Klebsiella* in drinking water emanating from redwood tanks. *Appl. Environ. Microbiol.* 33:893-905, 1977.

22. Reilly, J.K. and Kippin, J.S. Relationship of bacterial counts with turbidity and free chlorine in two distribution systems. *J. AWWA.* 75(6):309-312, 1983.

23. Camper, A.K., LeChevallier, M.W., Broadaway, S.C., and McFeters, G.A. Growth and persistence of pathogens on granular activated carbon filters. *Appl. Environ. Microbiol.* 50(6):1378-1382, 1985.

24. Camper, A.K., Broadaway, S.C., LeChevallier, M.W., and McFeters, G.A. Operational variables and the release of colonized granular activated carbon particles in drinking water. *J. AWWA.* 79(5):70-74, 1987.

25. Levy, R.V., Hart, F.L., and Cheetham, R.D. Occurrence and public health significance of invertebrates. *J. AWWA.* 78(9):105-110, 1986.

26. Chang, S.L., Berg, G., Clarke, N.A., and Kabler, P.W. Survival and protection against chlorination of human enteric pathogens in free-living nematodes isolated from water supplies. *Jour. Trop. Med. Hyg.* 9:136-142, 1960.

27. Silverman, G.S., Nagy, L.A., and Olson, B.H. Variations in particulate matter, algae, and bacteria in an uncovered finished drinking water reservoir. *J. AWWA.* 75(4):191-195, 1983.

28. Nagy, L.A., Kelly, A.J., Thun, M.A., and Olson, B.H. Biofilm composition, formation and control in the Los Angeles aquaduct system. *In:* Technology Conference Proceedings, WQTC-10, 1982, The Laboratory's Role in Water Quality, American Water Works Association, Denver, Colorado. pp. 141-160, 1983.

29. Donlan, R.M. and Pipes, W.0. Pipewall biofilm in drinking water mains. *In:* Technology Conference Proceedings, WQTC-14, 1986, Advances in Water Analysis and Treatment, American Water Works Association, Denver, Colorado. pp. 637-660, 1987.

30. Tuovinen, O.H., Button, K.S., Vuorinen, A., Carlson, L., Mair, D.M., and Yut, L.A. Bacterial chemical and mineralogical characteristics of tubercles in distribution pipelines. *J. AWWA.* 72(11):626-635, 1980.

31. Allen, M.J. and Geldreich, E.E. Distribution line sediments and bacterial regrowth. *In:* Technology Conference Proceedings, WQTC-5, 1977, Water Quality in the Distribution System, American Water Works Association, Denver, Colorado, 1978.

32. Allen, M.J., Taylor, R.H., and Geldreich, E.E. The occurrence of microorganisms in water main encrustations. *J. AWWA.* 72:616-625, 1980.

33. Ridgway, H.F. and Olson, B.H. Scanning electron microscopy evidence for bacterial colonization of drinking water distribution systems. *Appl. Environ. Microbiol.* 41(1):274-287, 1981.

34. Van der Kooij, D., Visser, A., and Hijnen, W.A.M. Determining the concentration of easily assimilable organic carbon in drinking water. *J. AWWA*. 74(10):540-545, 1982.

35. LeChevallier, M.W., Lee, R.G., and Moser, R. Disinfection of bacterial biofilms. American Water Works Service Company, Report Authorization 270, Formula 40-00. Belleville, IL, 1987.

V. Sampling and Analytical Methods

Environmental Sampling for Waterborne Pathogens: Overview of Methods, Application Limitations and Data Interpretation

by:Joan B. Rose
Department of Environmental
and Occupational Health
College of Public Health
University of South Florida
Tampa, FL 33612
(813) 974-3623

Introduction

Two key events that trigger the development of detection methods for pathogenic microorganisms in water are the recognition of an agent as a human pathogen and the waterborne transmission of the agent.

Although *Cryptosporidium* was first described in 1907 (1) and *Giardia*, in 1859 (2), the recognition of waterborne cryptosporidiosis and giardiasis in 1985 and 1965 came only after they had formally been established as human pathogens in 1980 and 1960, respectively. Similarly, *Yersinia enterocolitica* was described in 1923 and recognized as a pathogen in 1963, but the first waterborne outbreak was not documented in the United States until 1980 (3). *Campylobacter* was initially identified and described as a human pathogen in 1971, and by 1978 a waterborne outbreak had been identified (Table 5.1.1).

Many of the enteric viruses have long been recognized as causing waterborne disease, and methods have been developed and used for their detection in wastewater since 1945 and in drinking water, since 1960 (5). In 1913, Sawyer first identified the polio virus in human stools (6), and during the 1940s much debate ensued regarding the potential for waterborne transmission of this and related enteroviruses, such as echoviruses and coxsackieviruses. There was convincing evidence that the hepatitis A virus (HAV) was transmitted through water, but methods for its detection in the environment were not developed until the late 1970s. The rotavirus and Norwalk virus were identified in 1973 in human stools and in 1968 during an outbreak, respectively (7). Methods for rotavirus detection in the environment were under development by 1978 (8), concurrently with

Table 5.1.1. Events Leading to the Recognition of Waterborne Disease for Selected Pathogens

Microorganism	First described	Recognized as human pathogen	Waterborne outbreaks First documented	 Total number[a]
Cryptosporidium	1907	1980	1985	2[b]
Giardia	1859	1960	1965	95
Campylobacter	1971	1971	1978	11
Yersinia enterocolitica	1923	1960	1980	2

[a] In the United States up to 1985 (4).
[b] In the United States up to 1987.

the first report of a suspected waterborne outbreak (9). Methods for cultivating the Norwalk virus in the laboratory are not currently available. In addition, there are over 100 different types of enteric viruses that may be present in wastewater such as adenoviruses and caliciviruses that are not well studied.

The causes of most diarrhea in the clinical laboratory are not identified (10). Interestingly, identifiable agents in waterborne outbreaks follow a similar pattern.

A number of reviews have compiled data on outbreaks in Scotland, Sweden, the United Kingdom, and the United States (Table 5.1.2 [3,11-13]). The reports covered as few as 9 years in Sweden to as many as 65 years in the United States. The total number of outbreaks divided by the years of review gave a range of 0.7 to 3.5 outbreaks per year in Europe, while the United States averaged 24 outbreaks per year. In 23 to 59 percent of the outbreaks no causative agent was identified, perhaps because the causative agent had not yet been recognized as a human pathogen. Such was the case with *Cryptosporidium* prior to 1980. Another problem may be inadequate investigation of the outbreak due to inappropriate sampling or inadequate analysis.

Table 5.1.2. Reported Outbreaks of Waterborne Disease up to 1987 (2,11-13)

Location	Outbreaks Years of Reporting	 Total	 Parasites	 Viruses	 Bacteria	Unknown Etiology
Scotland	1955-1987	57	4	4	4	16
Sweden	1975-1984	32	1	2	11	19
United Kingdom	1937-1986	34	3	[a]	14	8
United States	1920-1985	1587	96	97	622	699

[a] The eight unknown were classified as possibly viral.

Bennett *et al.* (14) in a recent review of disease in the United States summarized the data for 1985. They collected data on the total incidence of enteric infections in the United States for the year 1985 and estimated the number of cases acquired through water as a proportion of overall morbidity. They estimated 940,000 cases of waterborne disease with 95 percent due to *Campylobacter*, Norwalk virus, enteric *Escherichia coli*, *Giardia*, and *Salmonella* (Table 5.1.3). For comparison only 1,561 cases of the disease occurred in the 16 documented waterborne outbreaks in the same year (15).

Table 5.1.3. Estimated Cases of Waterborne Disease in the United States for 1985

Microorganism	No. of Enteric Infections from all Cases (14)	Enteric Infections Felt to be Transmitted by Water (14)		Cases Reported from Waterborne Outbreaks (15)
		Percent	Cases	
Campylobacter	2,100,000	15	315,000	169
E. coli	200,000	75	150,000	[a]
Salmonella	2,000,000	3	60,000	60
Giardia	120,000	60	72,000	741
Norwalk virus	6,000,000	5	300,000	[a]

[a] None reported in 1985.

Current documentation of outbreaks does not begin to identify the impact of waterborne disease in the United States. It is essential that methods are developed and utilized for evaluating pathogen occurrence in water as well as in humans. Such methods will provide valuable information on potential risks of waterborne disease and the occurrence of various waterborne agents, particularly during catastrophic conditions (floods, treatment failures, contamination events, and outbreaks), which can then be used to implement strategies to protect against waterborne disease transmission.

Overview of Methods to Recover Pathogens from Water

Filtration procedures have been developed to recover and concentrate viruses and parasites from large volumes of water (100 to 1,000 L) and bacteria from smaller volumes (1 L). These procedures are routinely followed by microscopic procedures, tissue culture techniques, and bacteriological culture methods for the detection of the microorganisms. Finally, more sophisticated techniques (immunological, biochemical) may be needed to specifically identify a pathogen.

Early attempts in the 1940s to recover viruses from water samples involved the use of gauze pads; it was not until between 1976 and 1978 that electronegative and electropositive filters were designed specifically to recover enteric viruses from water (16). With a field method and tissue culture techniques established for the enteroviruses (polioviruses, coxsackieviruses, and echoviruses), documentation of published reports on viral contamination in drinking water dramatically increased (5). Specialized techniques for the detection of rotavirus and HAV in cell culture were developed, and the first updated isolations of these viruses from drinking water soon followed between 1982 and 1984 (17-19).

The methods to recover enteric protozoa began with *Giardia* during the first waterborne outbreak in 1965 (20). However the yarn-wound filters which could be used readily to sample large volumes of water were not introduced until the mid 1970s (21). Sauch, in 1985, made another significant contribution when she developed a technique that utilized a specific antibody to the *Giardia* cyst on membrane filters for detection with fluorescence microscopy (22). By 1985 a similar method had been developed for detecting *Cryptosporidium* oocysts in water (23,24).

Culture methods developed for bacteria in the clinical laboratory, using selective and differential media for growth and identification, were used similarly for environmental samples. The techniques for recovering these organisms from water were developed by individual groups of investigators during specialized surveys (5,25,26). There has been no single approach or strategy to evaluate or to optimize recovery efficiencies for the bacteria (including filter type, sample volumes, resuscitation procedures). Generally, the 100-mL sample volume used for coliform analysis was deemed not to be sufficient; 1,000 mL appeared more appropriate and could be collected with various types of filtration systems (membrane filters, depth filters). Recent developments have utilized naldixic acid and acridine orange to microscopically enumerate, by an epifluorescence procedure, bacteria in water samples which may be viable but nonculturable (27).

Current Status, Limitations, and Future of Methods for Detecting Microorganisms in Water

The detection methods for viruses, parasites, and bacteria in water samples are summarized in Table 5.1.4. Two major problems are common to all the methods. The first deals with recovery efficiencies, which may range from 1 percent to 70 percent, depending on the water conditions and quality. Virus and parasite recovery may be good from waters of drinking water quality; however, disinfection of the water may lead to greatly underestimating the stressed bacteria that are no longer cultivatible but still capable of initiating infections (28). The second problem is that the time required to process the sample and obtain the results could range from 2 to 80 days.

Within each group of microorganisms there are also unique problems. Although the enteric viruses often are referred to

Table 5.1.4. Routine Methods for Recovery and Detection of Viruses, Parasites, and Bacteria in Water

Microorganism	Filter Type	Recovery	Sample Processing	Detection
Viruses	Electronegative or electropositive cartridge filters	Eluted with beef extract	Organic precipitation	Tissue culture
Parasites	Yarn-wound cartridge filters	Washed from filter	Centrifugation density gradients	Microscopic immunofluorescence
Bacteria	Membrane filters	Placed in enrichment media	Selective media	Biochemical testing, serotyping

collectively, they form a very diverse group of microorganisms. There are over 100 types of viruses from humans that could be found in sewage; however, methods have been developed and are generally utilized to recover and identify only a few of the enteroviruses. Tissue culture systems are not available for the Norwalk-like viruses or for many of the adenoviruses, and are not routinely used for HAV or the rotaviruses. Therefore, little is known about the occurrence of these important viral agents and their potential for causing waterborne disease.

Cryptosporidium and *Giardia* can be specifically identified using monoclonal antibodies. However, the viability of the oocysts and cysts is unknown in environmental samples. This lack of knowledge leads to ambiguity in interpreting their health significance, particularly when the organisms are identified in treated waters. (See Table 5.1.5 for a summary of methods limitations.)

Table 5.1.5. Limitations of the Methods for Viruses, Parasites, and Bacteria

Group of Microorganism	Major Limitation
Viruses	Methods are not available for many important viruses (Norwalk).
Parasites	Viability of oocysts and cysts is unknown.
Bacteria	Methods are not quantitative.
Common to all	Recoveries can be poor.

Quantitative results are normally obtained for viruses and parasites, which are particularly beneficial when evaluating the potential health risks. Qualitative data are most often reported for the bacteria if a preenrichment step is utilized. A most probable number approach can also be used to estimate bacterial concentrations, but it requires a tremendous amount of work. Regardless of approach, without a preenrichment step, the bacteria may not be detected or may be greatly underestimated.

Methods development is generally a slow process, but new technology is being evaluated for detecting waterborne pathogens. Approaches using molecular biology, such as gene probes, have been utilized in the diagnosis of disease and are gradually being tested for environmental samples (29-31). Gene probes are pieces of nucleic acids (DNA or RNA) which can bind to specific microorganisms and be detected with radioactive or enzyme-linked markers. Gene probe methods are rapid and specific and could be used for pathogens such as Norwalk virus. Although it will take many years to develop and define the limits of appropriate methods for environmental samples, gene probes will be part of the future of available diagnostic tests.

Investigations of Waterborne Outbreaks

In most waterborne outbreak investigations, the etiological agent is not recovered from the suspected water source. Often environmental microbiologists are not a part of the investigative team; appropriate equipment is not available; or samples are collected too late in the outbreak, missing the contamination event. The few investigations that have reported detecting the suspected pathogen in the water have contributed greatly to the documentation and evaluation of the outbreak.

During an outbreak of gastroenteritis in Georgetown, Texas (population 13,000), virus samples were collected (17). The outbreak began June 1, cases peaked June 13, and samples were collected from the sewage and ground water supply on June 19. The epidemiological investigation reported a 79 percent attack rate, but no pathogen was identified in the fecal specimens. However, serological data demonstrated a recent exposure to the coxsackie B3 virus in five of seven individuals. Drinking water samples were positive for coxsackie B3 virus, coxsackie B2 virus, and HAV (Table 5.1.6).

Table 5.1.6. Viral Contamination of the Ground Water in the Georgetown Outbreak

No. of Positive Samples/No. of Samples Collected	Type of Sample	Viruses	Average pfu/100 L[a]
5/5	Sewage	CB3, CB2, HAV	4,580
2/3	Well	Rotavirus CB3, CB2, HAV	5.3
1/3	Tap water	CB3	0.33

[a] Plague-forming units for coxsackieviruses (CB2, CB3).

Although the outbreak had officially ended June 23, by July 15, 29 cases of hepatitis were reported. Unfortunately, because the methods for detecting HAV from water required weeks to obtain results, prophylactic treatment of individuals in the population at risk was not possible.

Data on the environmental occurrence of the pathogens and attack rates have also been evaluated for waterborne outbreaks of giardiasis and cryptosporidiosis (Table 5.1.7) (32). Of five outbreaks investigated, *Giardia* cyst levels ranged from 0.87 to 21/100 L, and attack rates ranged from 0.5 to 16 percent. Generally the higher cyst levels corresponded with the higher attack rates. *Cryptosporidium* oocyst levels were much higher (63/100 L) during the outbreak in Carrollton, Georgia (33), which resulted in a 40 percent attack rate and affected 13,000 people. Surveys during periods when outbreaks were not occurring have reported that an average of 17 percent of drinking water samples contained *Giardia* cysts or *Cryptosporidium* oocysts at levels of 0.19 and 0.11/100 L, respectively (Table 5.1.7). This low contamination level may be contributing to sporadic cases of disease in the community, but serological surveys are necessary to confirm this hypothesis, as these cases are not being detected through the current waterborne disease surveillance system.

Table 5.1.7. *Cryptosporidium* Oocyst and *Giardia* Cyst Contamination in Drinking Water during Waterborne Outbreaks

Giardia Cysts/100 L (Mean Levels in Water)		*Cryptosporidium* Oocysts/100 L (Mean Levels in Water)	
From Five Outbreaks[a]	Survey of Other Potable Waters	Carrollton Outbreak[b]	Survey of Other Potable Waters
11	0.19	63	0.11

[a] Mean.
[b] Mean of nine samples.

Recommendations for Environmental Sampling

The collection of microbial samples is one of the most important steps in obtaining accurate results, particularly during an outbreak. Considerations for sampling include proper collection procedures, timing of sample collection, sampling sites, replicate samples, sample volumes, proper storage, and handling and transportation of the samples. During an outbreak situation, it is imperative that environmental samples are collected as soon as an outbreak is suspected (Figure 5.1.1).

The time of recognition of an outbreak depends on the incubation period of the disease from a specific organism. Outbreaks having short incubation periods are more likely to be reported and investigated quickly because they are easier to recognize. Also, in the event of a

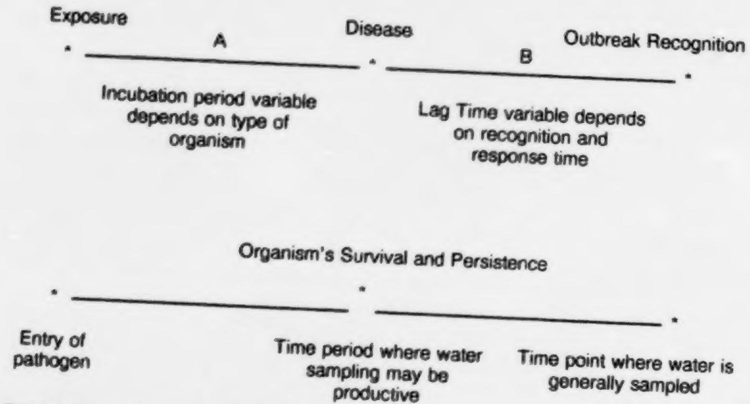

Exposure
A
Disease
B
Outbreak Recognition
Incubation period variable depends on type of organism
Lag Time variable depends on recognition and response time
Organism's Survival and Persistence
Entry of pathogen
Time period where water sampling may be productive
Time point where water is generally sampled

Figure 5.1.1. Events in an outbreak.

short incubation period, investigators will probably be able to respond to the outbreak faster, as the organism is more likely to still be present in the water system. Lag time, however, is dependent primarily on human factors, such as interest in the investigation, rather than the length of the incubation period.

Many bacteria have a relatively short incubation period (2 to 5 days). The incubation period of many protozoans and viruses can be much longer than for bacteria, i.e., 1 or 6 weeks for *Cryptosporidium* and HAV, respectively. The amount of contamination and the lag time for recognition will influence the ability to recover and detect the bacteria, depending on their survival characteristics and environmental transport patterns. Bacteria can die off within a few days after the contamination event; and although viruses, and particularly parasites, have a greater ability to survive and remain in a system, even an immediate response may miss the exposure event.

Because it may be impossible to sample the water during the contamination event, it is very important to take enough samples at locations where pathogen residuals may be identified. If a contaminated well is suspected, then a minimum of three replicate samples per well should be collected. Seven to 10 samples should be collected from a suspected distribution system, particularly at dead-end mains where microorganisms may accumulate. In some cases, investigators may be interested in sampling the sewage to further document infection in the population and to demonstrate at a later time that the outbreak is over.

Samples for viruses, parasites (minimum volume of 400 L), and bacteria (2-L volumes) all should be collected, if possible, unless there is some immediate indication that one type of pathogen is responsible. Sample analysis for viruses can wait if the filter concentrate is frozen. Parasite samples may be stored at 4°C in 3.7 percent formaldehyde. Bacterial samples should be assayed within 24 hours and stored at 4°C until that time. All filters should be stored at 4°C until processed.

Finally, any laboratory can develop an action strategy and obtain the proper equipment and training for environmental sampling of microorganisms. This strategy could routinely be included in any emergency plan or specific study.

Once samples are collected, they can be shipped to other laboratories with the necessary capabilities for analysis.

Summary

As the role of contaminated water in the transmission of viruses, parasites, and bacteria was being elucidated, methods were under development for the detection of these microorganisms in water. Filtration methods have been developed for both the viruses and parasites (volumes of 400 L). The samples are then assayed on cell culture and using microscopic immunofluorescence techniques for viruses and parasites, respectively. For bacteria such as *Campylobacter*, filtration methods are also utilized (1-L samples), followed by enrichment and selective culture techniques.

At issue with any of these methodologies is the effect of water quality on the recovery efficiencies. Other limitations include 1) lack of cell culture techniques for viruses such as the Norwalk virus, 2) inability to assess viability of the protozoa and 3) the detection of noncultivatable and stressed bacteria.

Recommendations for environmental sampling include:

- Immediate collection of the water samples upon recognition of an outbreak
- Collection of a minimum of 400 L for viruses and parasites and 1 L for bacteria
- Collection of 3 replicate samples of an individual well or 7 to 10 samples of treated water (distribution system and dead-end mains)
- Collection and assay for a broad range of microorganisms
- Obtaining proper equipment and training

References

1. Tyzzer, E.E. A sporozoan found in the peptic glands of the common mouse. *Proc. Soc. Exp. Biol. Med.* 5:12-13, 1907.
2. Feachem, R.G., Bradley, D.H., Garellick, H., and Mara, D.D. Sanitation and Disease Health Aspects Excreta and Wastewater Management. John Wiley and Sons, New York, NY, 1983.
3. Craun, G.F. Waterborne diseases in the United State,s. CRC Press, Boca Raton, FL, 1986.
4. Craun, G.F. Surface water supplies and health. *J. Amer. Water Works Assoc.*, 80:40-52, February, 1988.
5. Rose, J.B. and C.P. Gerba. A review of viruses in drinking water. *In:* Current Practices in Environmental Engineering. Vol. 2. pp. 119-143, 1986.
6. Sawyer, W.A. An epidemiological study of poliomyelitis. *Amer. J. Trop. Dis. Prev. Med.* 3:164-175, 1915.
7. Gerba, C.P., Rose, J.B. and Singh, S.N. Waterborne gastroenteritis and viral hepatitis. *CRC Critical Rev. in Environ. Control.* 15:213-236, 1985.
8. Ramia, S. and Sattar, S.A.. Concentration of seeded Simian rotavirus SA-11 from potable waters by using talc-celite layers and hydroextraction. *Appl. Environ. Microbiol.* 39:493, 1980.
9. Lycke, E., Bloomberg, J., Berg, G., Eriksson, A., and Madsen, L. Epidemic acute diarrhoea in adults associated with infantile gastroenteritis. *Lancet.* 2:1056, 1978.
10. Flewett, T.H., Beards, G.M. Brown, D.W.G. and Sanders, R.C. The Diagnostic Gap in Diarrhoea Viruses. G. Bock and J. Whelan (eds.), John Wiley and Sons Ltd., Chichester, U.K., 1987. pp. 238-249, 1987.
11. Anderson, Y., and Stenstrom, T.A. Waterborne outbreaks in Sweden – causes and etiology. *Water Sci. Tech.* 18:185-190, 1986.
12. Benton, C., Forbes, G.I. Paterson, G.M., Sharp, J.C.M. The incidence of waterborne and water associated disease in Scotland from 1945-1987. *In*: Developments at the 14th International Conference on Water Pollution Research, Brighton, England. U.S. EPA, Cinncinati, OH. p. 157, 1989.
13. Galbraith, N.S., Barrett, N.J., and Stanwell-Smith, R. Water and disease after Croydon: a review of waterborne and water-associated disease in the UK, 1937-86. *J. Instit. of Water and Environmental Management.* 1:7- 21, 1987.

14. Bennett, J.V., Homberg, S.D., Rogers, M.F., and Solomon, S.L. Infectious and Parasitic Diseases. *Amer. J. of Preventative Medicine.* 3:102-114, 1987.

15. Water-related disease outbreaks. *Morbidity Mortality Weekly Report.* 37:15-24, 1988.

16. Gerba, C.P. and Goyal, S.M. Method in Environmental Virology. Gerba, C.P. (ed). Marcel Dekker, Inc., New York, 1982.

17. Hejkal, T.W., Keswick, B., LaBelle, R.L., Gerba, C.P., Sanchez, Y., Dressman, G., Hafkin, and Melnick, J.L. Viruses in a community watersupply associated with an outbreak of gatroenteritis and infectious hepatitis. *J. Amer. Water Works Assoc.* 74:318-321, 1982.

18. Keswick, B.H., Gerba, C.P., DuPont, H.L., and J.B. Rose. Occurrence of viruses in treated drinking water. *Appl. Environ. Microbiol.* 47(6):1290-1294, 1984.

19. Sobsey, M.D., Oglesbee, S.E., and Waite, D.A. Detection of hepatitis A virus (HAV) in drinking water. *In:* G.N. Vyas, Dienstag, J.L., and Hoofnagle, J.H. (ed.), Viral Hepatitis and Liver Disease. Grune and Stratton, Inc., 1984.

20. Moore, G.T., Cross, W.M., McGuire, D., Mollohan, C.S., Gleason, N.W., Healy, G.R., and Newton, L.H. Epidemic giardiasis at a ski resort. *N. Engl. J. Med.* 281:402-407, 1969.

21. Jakubowski, W. and J.C. Hoff. Waterborne transmission of giardiasis. Report No. EPA-600/9-001. U.S. Environmental Protection Agency. Cincinnati, OH, pp. 104-126, 1979.

22. Sauch, J.F. Use of immunofluorescence and phase-contrast microscopy for detection and identification of *Giardia* cysts in water samples. *Appl. Environ. Microbiol.* 50(6):1434-1438. 1985.

23. Musical, C.E., Arrowood, M.J., Sterling, C.R., and Gerba, C.P. Detection of *Cryptosporidium* in water using polypropylene cartridge filters. *Appl. Environ. Microbiol.* 53:687-692, 1987.

24. Rose, J.B., Kayed, D., Madore, M.S., Gerba, C.P., Arrowood, M.J., and Sterling, C.R. Methods for the recovery of *Giardia* and *Cryptosporidium* from environmental waters and their comparative occurrence. *In:* P. Wallis and B. Hammond (ed), Advances in Giardia Research. Univ. Calgary Press. pp. 205-209, 1988.

25. Vogt, R.L., Siurs, H.E., Barrett, R., Feldman, R.A., Dickinson, R.J., and Witherell, L. *Campylobacter* enteritis associated with contaminated water. *Ann. Intern. Med.* 96:292, 1982.

26. Schiemann, D.A. Isolation of *Yersinia enterocolitica* from surface and well waters in Ontario. *Canadian Jour. of Microbio.* 24:1048, 1978.

27. Roszak, D.B., and Colwell, R.R. Metabolic activity of bacterial cells enumerated by direct viable count. *Appl. Environ. Microb.* 53:2889-2983, 1987.

28. LeChavallier, M.W., Shiemann, D.A., and McFeters, G.A. Factors contributing to the reduced invasiveness of chlorine-injured *Yersinia enterocolitica. Appl. and Environ. Microbiol.* 53:1358, 1987.

29. Richardson, K.J., Margolin, A.B., and Gerba, C.P. A novel method for liberating viral nucleic acid for assay of water samples with cDNA probes. *J. Virol. Methods.* 2:13-21, 1988.

30. Somerville, C.C., Knight, I.T., Straube, W.L., and Colwell, R.R. Simple, rapid method for direct isolation of nucleic acids from aquatic environments. *Appl. Environ. Microb.* 55:548-554, 1989.

31. Steffan, R.J. and Atlas, R.M. DNA amplification to enhance detection of genetically engineered bacteria in environmental samples. *Appl. Environ. Microb.* 54:2185-2191, 1988.

32. Rose, J.B. *Cryptosporidium* in water; risk of protozoan waterborne transmission. Report for American Association of Advancement of Science/Environmental Protection Agency Science and Engineering Fellow. 1988.

33. Hayes, E.B., Matte, T.D., O'Brien, T.R., McKinley, T.W., Logsdon, G.S., Rose, J.B., Ungar, B.L.P., Word, D.M., Pinsky, P.F., Cummings, M.L., Wilson, M.A., Long, E.G., Hurwitz, E.S., and Juranek, D.D. Contamination of a conventionally treated filtered public water supply by *Cryptosporidium* associated with a large community outbreak of cryptosporidiosis. *New England Journ. Med.* 320:1372-1376, 1989.

Bacteriologic Analysis of Clinical Specimens in Waterborne Disease Outbreaks

by: Julie Parsonnet, M.D.
Division of Geographic Medicine
HRP Building, Room 109
Stanford University
Stanford, California 94062
(415) 725-4561

This chapter will describe bacteriologic laboratory support for clinical samples, focusing on bacterial enteric outbreak investigations. It will also briefly discuss nonenteric waterborne diseases.

In the years 1971 to 1985, 61 waterborne disease outbreaks caused by bacterial agents were reported to the Centers for Disease Control (CDC). Bacteria implicated included *Shigella, Campylobacter*, nontyphoidal *Salmonella, Salmonella typhi, Vibrio cholerae*, and *Yersinia enterocolitica*. To determine the agents in these outbreaks, a combination of basic epidemiologic and microbiologic principles were applied.

Identifying Etiologic Agents

Human Specimens

The primary principle in the microbiologic investigation of an outbreak is that human pathogens are found in people. One will sometimes find suspicious organisms in environmental samples, which suggest causation, but the key to identifying an etiologic agent is to look in the patient rather than in the environment. For instance, *Yersinia* species are not infrequently found in environmental samples but one cannot assume that they have caused diarrheal disease unless they are also found in the stool specimens of the ill persons.

In the bacteriologic analysis of human specimens three outcomes are possible. First, quite commonly one may find nothing. Second, one may find a *known* pathogen, such as *Salmonella* or *Shigella*. In that case, if the clinical illness seen in the outbreak is typical of that caused by the organism, the etiologic agent has been identified. A third outcome, which occurs fairly frequently, however, is that a *suspicious* organism that is not necessarily a pathogen (e.g., *Yersinia enterocolitica*), is identified. In such instances, the organism must be statistically more common in specimens from ill persons (cases) than from well persons (controls) to be considered the outbreak etiologic agent. An organism equally common in both cases and asymptomatic controls must be presumed to be unassociated with the outbreak illness.

Environmental Samples

Of secondary importance in waterborne outbreak investigations is the culture of environmental samples. In general, finding a known pathogen or a possible pathogen in water is not sufficient to incriminate the organism as the cause of the outbreak. The agent has to be isolated from human specimens, as well. Thus, if *Yersinia* is found in a water source but not in human stool, it cannot be assumed that the organism caused the illness. There are some exceptions to this rule; diseases that have very specific clinical presentations may be diagnosed from environmental samples without confirmatory human specimens. Examples of agents that can cause such pathognomonic symptoms are *Staphylococcus aureus* enterotoxin and *Clostridium botulinum* toxin. If either of these agents are found in epidemiologically suspect food vehicles, investigators would assume that they caused the associated specific and dramatic human illnesses, even in the absence of confirmatory human specimens.

In summary, the three routes to identifying the etiologic agent of an outbreak are:

1. A known pathogen (e.g., *Shigella*) that causes symptoms compatible with the outbreak illness is found in human specimens.
2. A possible pathogen (e.g., *Yersinia*) is found statistically more commonly in specimens from ill persons than in those from well persons.
3. A known pathogen that causes pathognomonic symptoms is found in the implicated environmental source.

Specimen Collection

Because clinical specimens are the key to identifying the etiologic agent of an outbreak, the most important part of the laboratory investigation is proper specimen collection. In an outbreak investigation, regardless of outbreak size, 10 specimens from ill persons and 10 specimens from well persons usually suffice. Upon receiving a fresh stool sample, an investigator should immediately place two sterile swabs of stool in Cary-Blair or similar transport medium and refrigerate them along with the remainder of the whole stool sample. The swabs can remain refrigerated for up to 48 hours and the whole stool for 4 hours before delivery to the laboratory; if longer delays are anticipated, samples should be frozen, preferably at -70°F, although -20°F will suffice. This protocol eliminates frequent freezing and thawing of the whole stool sample, minimizing compromise of the specimens.

If the clinical illness suggests a viral or parasitic etiology, whole stool specimens will need to be divided into aliquots so they can be handled appropriately. Our current practice at CDC is to ask that the aliquots held for viral examination be refrigerated indefinitely without freezing; the aliquot for parasitic analysis should be transferred to an appropriate parasitic transport medium.

The swabs should be used for routine bacterial analysis; the whole stool can be reserved for necessary special studies. Stools from ill persons should be analyzed first. If those cultures yield no likely agent, the specimens from well persons need not be tested.

Bacteriologic Study Techniques

The microbiologic studies performed in an outbreak investigation should be tailored to the clinical illness and the suspected organism involved. The analyses themselves can be divided into three categories:

1. Bacterial culture and the use of epidemiologic markers
2. Pathogenicity assays
3. Serologic tests for antibacterial antibodies.

Tests from one or all of these methods may be applied to specimens from any given outbreak (see Table 5.2.1).

Table 5.2.1. Bacteriologic Techniques in the Investigation of a Waterborne Outbreak

1. Bacterial cultural and epidemiologic markers
    a. Routine culture and biochemical identification
    b. Serogroup/serotype
    c. Antimicrobial resistance pattern
    d. Plasmid profile
    e. Isoenzyme type and/or biotype
2. Pathogenicity assays
    a. Toxin assays
    b. Adhesiveness, invasiveness assays
3. Serologic tests

Bacterial Culture and Epidemiologic Markers

Routine Culture

Routine bacterial culture of stool is the most common technique used to detect an etiologic agent in enteric diseases and is essential to any investigation. Culture techniques must be designed to include all suspect organisms. In many outbreaks, however, culture alone is not sufficient to trace the cases and the source. Tests which more specifically identify the strain, such as serogrouping or serotyping, are required.

Serogrouping and Serotyping

Serogrouping classifies the enterobacteria using one antigen, usually the heat-stable somatic O antigen. Serotyping identifies an organism more specifically by defining many antigens on the bacterial surface. As such, it requires a more complex collection of antisera and is usually restricted to laboratories in major medical centers, state public health departments, and reference centers such as the Centers for Disease Control (CDC). Serotyping is helpful for classifying many organisms, *Salmonella* chief among them.

Researchers have thus far identified approximately 2,000 different serotypes of *Salmonella* with disparate environmental and clinical prevalences. A steady increase in the *enteriditis* serotype was recognized in 1984, when investigations by state health departments in conjunction with CDC, U.S. Department of Agriculture, and U.S. Food and Drug Administration identified intact grade A shell eggs as the primary source of *Salmonella enteriditis*. This discovery is resulting in major changes in the egg industry (1,2). Without serotyping, the health risk would never have been uncovered.

Shigella strains demonstrate another example of the usefulness of serotyping. *Shigella* are classified into four serogroups, A through D; each of the first three groups is then subdivided into many serotypes. Serotype A1, the Shiga bacillus, is quite rare in the United States but causes severe, often life-threatening, illness. Serotyping by the state public health laboratories recently enabled researchers to identify an outbreak of serotype A1 in tourists and to identify the source as Cancun, Mexico. This strain is still being monitored using state reports of *Shigella* serotypes (3).

Escherichia coli is another organism for which serotyping has been incalculably helpful. Before the advent of serotyping, *E. coli* were usually considered to be nonpathogenic to the bowel. Now, it is apparent that certain serotypes are responsible for a wide diversity of gastrointestinal illness, from chronic diarrhea in children to severe hemorrhagic colitis and hemolytic uremic syndrome caused by verotoxigenic strains such as *E. coli* 0157:H7.

Antimicrobial Sensitivity

Antimicrobial sensitivity profiles can also be used to epidemiologically trace strains associated with an outbreak. Although organisms can rapidly change their antimicrobial sensitivity through plasmid exchange, many times the antibiograms remain remarkably stable. In 1986, a huge outbreak of *Shigella sonnei* among a gathering of the Rainbow family in North Carolina was traceable because the organism had a unique antibiotic sensitivity (4). Similarly, in 1983, *Salmonella newport* was traced from patients to hamburger meat from antimicrobially treated cattle because of the organism's unusual, multiresistant antibiotic pattern (5).

Plasmid Profile

A more sophisticated epidemiologic tool is plasmid DNA electrophoresis. This technique dissects the genetic composition of bacterial strains and enables researchers to identify likely clones. Using these molecular techniques, identical *E. coli* 0157:H7 strains were identified in patients and in implicated hamburger meat from the first recognized outbreak of hemorrhagic colitis in 1982 (6). Similarly, illness caused by *Salmonella newport* was traced by plasmid profile to ground beef (5).

Isoenzyme Type and/or Biotype

Other epidemiologic markers are helpful but less frequently used. Biotyping is used for *Vibrio cholera* and *Yersinia* species. Isoenzyme typing is the preferred strain identification technique for *Legionella pneumophila*. Phage typing, frequently used for *Staphylococcus aureus*, is also used for *Salmonella typhi* and more recently for *Salmonella enteritidis*.

Pathogenicity Assays

With time, researchers of outbreaks have become increasingly sophisticated in their understanding of disease mechanisms. This has aided immeasurably in identifying new pathogens. However, even if an organism is epidemiologically linked with illness, investigators will be skeptical about causality unless a mechanism for disease has, or can be, identified with a pathogenicity assay.

Toxin Assays

One way to use pathogenicity assays is in looking for an illness mediator, such as a toxin. Toxins are identified in several ways. Although bioassays are slowly becoming obsolete, they are still used for identifying some bacterial pathogens. For example, enterotoxins are often identified using a rabbit ileal loop model, in which bacterial filtrate is injected into a blind ileal loop. Large volumes of fluid secretion indicate the presence of an enterotoxin. If the toxin's effects can be neutralized by a specific antitoxin, the presence of the toxin is confirmed.

More frequently, toxicity is assessed in cell culture. For example, shigatoxin is cytotoxic to Vero cells in culture. This effect is neutralized when shigatoxin antibody is added to the culture plates. The shiga-like toxins, or verotoxins, produced by some *E. coli* strains, have a similar effect on Vero cells.

DNA hybridization can be performed for any toxin for which the gene has been cloned, and is now done for cholera toxin, shigatoxin, shiga-like toxins, heat-labile and heat-stable enterotoxins (7). Enzyme-linked immunosorbent assays of culture filtrates have also been successfully used to detect these toxins (8, 9).

Adhesiveness/Invasiveness Assays

Other useful pathogenicity assays involve detection of inherently pathogenic traits of the bacteria themselves, such as their ability to adhere to cells or invade tissue. The methods used for detecting these traits are similar to those used to find toxin: bioassay, cell culture, and DNA hybridization techniques.

An example of a bioassay is the now infrequently used Sereny test, which detects bacterial invasiveness in guinea pig conjunctiva. Cell cultures and molecular techniques have largely displaced bioassays. For example, adherence can be detected by observing the effects of bacteria on HeLa cell cultures. In addition, the genes for adherence and invasiveness have been sequenced, allowing them to be detected with DNA hybridization (10).

Serologic Tests

Serum antibody tests are the final method for diagnosing outbreak-related disease. Serum antibacterial antibodies are most useful for organisms that are difficult to culture. One of the best examples of serologic testing for waterborne illness is immunofluorescent antibody testing for *L. pneumophila*. *Legionella* is often very difficult to isolate by routine sputum cultures. In a serologic test, a fourfold rise between acute- and convalescent-phase titers connotes an acute infection.

Investigators also rely on serodiagnosis when an acute outbreak has passed. In a study of *V. cholerae* infection along the American Gulf coast, vibriocidal antibody studies effectively demonstrated the extent of the problem (11). Investigators are currently evaluating serologic tests for *E. coli*- and *Shigella*-associated shigatoxin and shiga-like toxins.

Recent Technology

One experimental technique that holds great promise in many branches of microbiology is the polymerase chain reaction (PCR). PCR is able to detect organisms of very low frequency in laboratory specimens. In this technique, a gene or genetic sequence unique to a specific organism is cloned and a labeled probe is made using this sequence. DNA primers, sequences complementary to regions flanking the cloned gene, are then added to a clinical specimen. These primers bind to the regions adjacent to the cloned sequence and serve to attach DNA polymerase. By alternately warming and cooling a surrounding bath, the unique sequence can then be amplified thousands of times. This enhancement allows probe detection of what otherwise may have been an undetectably rare gene. Epidemiologic and diagnostic uses for this emerging technique may be numerous.

Over the last 10 years, many new bacterial pathogens have been identified using the microbiologic and epidemiologic methods described above. Among these are *E. coli* 0157:H7, *Campylobacter jejuni*, *V. vulnificus*, and *L. pneumophila*.

Although many of these can be identified using the routine culture and bacterial identification techniques available in any standard laboratory (Table 5.2.2), the more sophisticated tests now available in reference laboratories are enabling researchers to define the epidemiology of disease transmission on an increasingly specific molecular level.

Table 5.2.2. Microbiologic Techniques Used for Specific Bacterial Pathogen-Specific Bacterial Pathogens in Epidemiologic Investigations of Waterborne Disease Control[a]

Agent	*Culture*	*Antimicrobial Sensitivity*	*Serotype*	*Biotype*	*Plasmid Profile*	*Toxic Assay*	*Pathogenicity Assay*
Campylobacter	×		×	×			
Salmonella	×	×	×		×		
Shigella	×	×	×		×		
Yersinia	×		×	×			
E. coli	×	?	×	×	×	×	×
V. cholerae	×		×	×		×	

[a] Culture and antimicrobial sensitivity testing are widely available. Serogrouping and serotyping are available in many hospital laboratories and most state public health laboratories. Toxin assays and pathogenicity assays are available in specific research laboratories.

References

1. St. Louis, M.E., Morse, D.L., Potter, M.E. *et al.* The emergency of grade A eggs as a major source of *Salmonella enteritidis* infections: New implications for the control of salmonellosis. *JAMA.* 259:2103-2107, 1988.

2. Centers for Disease Control. *Salmonella enteritidis* infections and grade A shell eggs, United States, *MMWR.* 37:490, 495-6, 1988.

3. Centers for Disease Control. *Shigella dysenteriae* type 1 in tourists to Cancun, Mexico. *MMWR.* 37:465, 1988.

4. Centers for Disease Control. Nationwide dissemination of multiply resistant *Shigella sonnei* following a common-source outbreak. *MMWR.* 36:633-4, 1987.

5. Holmberg, S.D., Osterholm, M.T., Senger, K.A. *et al.* Drug-resistant salmonella from animals fed antimicrobials. *N. Engl. J. Med.* 311:617-22, 1984.

6. Riley, L.W., Remis, R.S., Helgerson, S.D. *et al.* Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308: 681-5, 1983.

7. Venkatesan, M., Buysse, J.M., Vandendries, E. *et al.* Development and testing of invasion-associated DNA probes for detection of *Shigella* spp. and enteroinvasive *Escherichia coli. J. Clin. Microbiol.* 26:261-6, 1988.

8. Svennerholm, A.M. and Holmgren, J. Identification of *Escherichia coli* heat-labile enterotoxin by means of a ganglioside immunosorbent assay (G_{M1}-ELISA) procedure. *Curr. Microbiol.* 1:19-23, 1978.

9. Lockwood, E.D. and Robertson, D.C. Development of a competitive enzyme-linked immunosorbent assay (ELISA) for *Escherichia coli* heat-stable enterotoxin (STa). *J. Immunol. Meth.* 75:295-307, 1984.

10. Nataro, J.P., Baldini, M.M., Kaper, J.B. *et al.* Detection of an adherence factor of enteropathogenic Escherichia *coli* with a DNA probe. *J. Infect. Dis.* 152:560-65, 1985.

11. Centers for Disease Control. Cholera in Louisiana – Update. *MMWR.* 35:687-8, 1986.

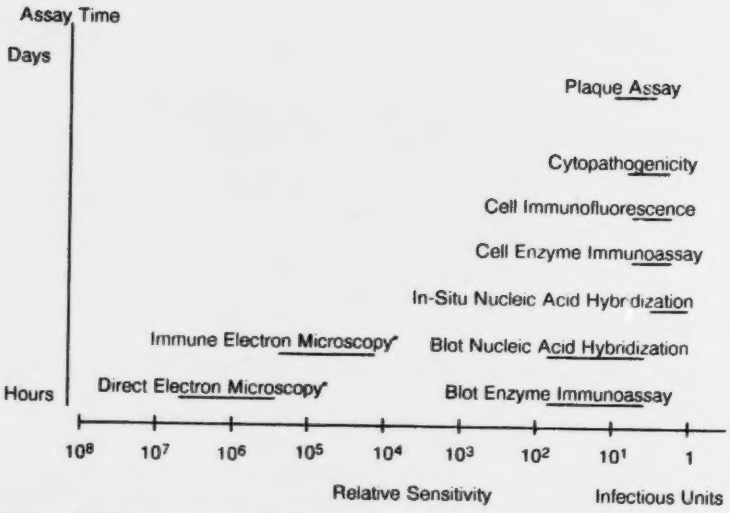
Clinical Laboratory Diagnosis of Enteric Viral Diseases

by: Christon J. Hurst, Ph.D.
Risk Reduction Engineering Laboratory
U. S. Environmental Protection Agency
Cincinnati, Ohio 45268
(513) 569-7331

Enteric viruses are those which replicate within, and are subsequently shed from, the human enteric tract. There are presently known to be more than 140 serologically distinct types, or serotypes, of human enteric viruses. Enteric viral infections are very often associated with stomach cramps, vomiting and diarrhea. Infections caused by some of the enteric viruses can spread from the enteric tract to other organs or tissues of the body. This can result in symptoms associated with abnormality or disfunction of these secondary sites, including fever, hepatitis, meningitis, and paralysis. Human enteric viruses occasionally cause outbreaks of waterborne human illness because they are transmitted by the fecal-oral route, meaning that they are passed from person to person via ingestion of fecally contaminated materials. Of the different human enteric viruses which have been recognized in association with waterborne illness, the most prominent are two causative agents of infectious hepatitis (human enterovirus 72, formerly hepatitis A virus; and hepatitis E virus, also called the enterically transmitted non-A non-B hepatitis virus) plus several causative agents of vomiting and diarrhea including the Norwalk virus.

The process of laboratory diagnosis or confirmation of a viral illness can be accomplished by demonstrating that collected specimens or samples of body materials contain either identifiable virus particles, viral structural components which can include proteins or nucleic acids, or specific antiviral antibodies that have been produced by the person's immunological defenses in response to infection. The types of specimens that are most commonly collected in association with suspected waterborne viral illnesses are stool solids and rectal swabs, which are primarily examined for the presence of whole viruses or viral specific antigens, and blood serum that is primarily examined for the presence of antiviral antibodies.

Many of the techniques used for detecting viruses and viral antigens are represented in Figure 5.3.1, where they are compared



* Physical Particles

Figure 5.3.1. Comparison of viral assay techniques.

with respect to relative sensitivity and also the length of time that is required for assay completion. This figure has been adapted from the referenced publication by Hurst and Stetler (1). The relative sensitivity ranges for these different techniques are indicated by horizontal bars that appear under the name of each technique. These sensitivity ranges are given in terms of quantitating physical virus particles for the electron microscopy techniques, and in terms of viral infectious units for the other assay techniques. Additional methodologies such as radioimmunoassay, ELISA (enzyme-linked immunosorbent assay), complement fixation, and hemagglutination can also be used for identifying either whole viruses or viral materials contained in collected specimens.

Serological assays are used to detect the presence of antiviral antibodies rather than the presence of virus particles or viral structural components. These serological tests utilize prepared viral particles, virally infected cells, or viral antigens as targets that are recognized by appropriate antibodies present in the patients serum. The serological assays can employ the same general methodology as used for viral detection, the difference being whether viral material or antibody represents the unknown component in a test procedure. The materials used for performing many of these techniques can be purchased from biomedical companies in the form of kits. Further details of these methods are provided in the referenced books edited by by Rose *et al.* (2) and Lennette *et al.* (3).

References:

1. Hurst, C. J. and R. E. Stetler, 1988. Recent advances in the detection of human viruses in drinking water. Pages 943-956 *in* Proceedings of the 15th. Water Quality Technology Conference, Baltimore, Maryland, November 15-20, 1987. American Water Works Association, Denver.
2. Manual of Clinical Laboratory Immunology (3rd. edition), 1986. Editors: N. R. Rose, H. Friedman, and J. L. Fahey. American Society for Microbiology, Washington.
3. Manual of Clinical Microbiology (4th. edition), 1985. Editors: E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. Jean Shadomy. American Society for Microbiology, Washington.

Clinical Diagnosis of Enteric Protozoans

by: Frank W. Schaefer, III
Environmental Systems Monitoring Laboratory
U.S. Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, Ohio 45268
(513) 569-7222

Enteric protozoan pathogens that have direct modes of transmission in humans include *Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum, Isospora belli*, and *Balantidium coli*. Of these, *B. coli* is rarely seen, even through it is ubiquitous and cosmopolitan in nature like the others. With the advent of the AIDS epidemic, the importance of the first four organisms has been reinforced.

Effective diagnostic procedures for these parasites require implementation of a number of steps. Appropriate sample collection must be followed by expeditious concentration, preservation, and staining techniques. Ultimately, microscopic reading must be carried out by personnel of considerable skill and experience. Furthermore, each parasite has unique qualities that prevent the utilization of universal procedures for all. Some parasites, like *Giardia*, are intermittent in cyst production or produce no cysts in some infected people. In addition to the necessity of doing multiple stool exams to overcome this characteristic, sometimes invasive procedures, like duodenal aspiration or biopsy, are required to make an accurate diagnosis. An overall approach for carrying out these steps has been published by the American Society of Parasitologists (1).

There is no consensus as to which of the published procedures for diagnosing these parasites is optimal. Each laboratory has a tendency to use those procedures with which they feel most comfortable, whether or not they are the most effective. Presently the Centers for Disease Control recommend the methods outlined by Melvin and Brook (2), while the American Society for Clinical Pathologists suggests procedures published by Ash and Orihel (3). The techniques listed in these volumes and numerous other credible sources, which space does not permit mentioning, are very labor intensive and require specialized skills which are rare to nonexistent in many clinical laboratories.

Attempts have been made to employ molecular and immunological procedures to simplify, expedite, and remove the high level of microscope skill required of the present methods. Papers have been published describing the use of monoclonal antibodies (4) to differentially stain the organisms of interest. Genetic probes (5) and enzyme-linked immunosorbent assays (6), which require only the genetic material or the parasitic fecal antigenic epitope, have been described as agents of potential clinical significance. Unfortunately, these methods are not a panacea, as problems such as cross reaction and sensitivity remain to be solved.

References

1. American Society of Parasitologists. 1977. Procedures for use in examination of clinical specimens for parasitic infections. *Journal of Parasitology* 63: 959-960.
2. Melvin, D.M. and Brooke, M.M. 1982. Laboratory Procedures for the Diagnosis of Intestinal Parasites, 3rd ed. HHS Publication No. (CDC) 82-8282.
3. Ash, L.R. and Orihel, T.C. Parasites: A Guide to Laboratory Procedures and Identification. American Society of Clinical Pathologists Press, Chicago. 328 p.
4. Sterling, C.R. and Arrowood, J.J. 1986. Detection of *Cryptosporidium* sp. infections using a direct immunofluorescent assay. *Pediatric Infectious Diseases* 5: S139-S142.
5. Samuelson, J., Acuna-Soto, R., Reed, S., Biagi, F., and Wirth, D. 1989. DNA hybridization probe for clinical diagnosis of *Entamoeba histolytica*. *Journal of Clinical Microbiology* 27: 671-676.
6. Janoff, E.N., Craft, J.C., Pickering, L.K., Novotny, T., Blaser, M.J., Knisley, C.V. and Reller, L.B. 1989. Diagnosis of *Gardia lamblia* infections by detection of parasite-specific antigens. *Journal of Clinical Microbiology* 27: 431-435.

Analysis of Water Samples for Bacterial Pathogens

by: Dr. Gerard N. Stelma, Jr.
Environmental Monitoring Systems Laboratory
Division of Microbiology Bacteriology Branch
U.S. Environmental Protection Agency
Cincinnati, OH 45268
(513) 569-7384

Introduction

Bacteria associated with waterborne illness outbreaks include two types of pathogens, those present in water as a result of pollution and some aquatic organisms that appear to be indigenous to drinking water distribution systems. The pathogens acquired from polluted waters are of fecal origin and are causes of gastrointestinal illness. Pathogens acquired from polluted waters include recently recognized species, such as *Campylobacter jejuni* and *Yersinia enterocolitica*, as well as classical enteric pathogens, such as *Salmonella* and *Shigella*. Most outbreaks of drinking water-associated gastroenteritis have resulted from human fecal pollution; however, animal fecal pollution is being increasingly implicated in outbreaks (1). The indigenous aquatic organisms are opportunistic pathogens that rarely infect healthy individuals but may cause serious (and sometimes fatal) infections in those who are compromised. *Legionalla pneumophila* is a good example of this type of pathogen. Not included in this review are pathogens associated with rare sporadic illnesses in the United States (e.g., *Leptospira* and *Vibrio cholerae*) or those normally associated with recreational water (e.g., *Pseudomonas aeruginosa*).

Investigations of waterborne outbreaks are complicated by 1) the low numbers and transient nature of the fecal pathogens in drinking water, 2) difficulties in detecting pathogens in water due to interferences by natural competing flora, and 3) the fastidious nutrient requirements of pathogenic bacteria (2). The methods used to isolate pathogens from potable water are modifications of methods used in clinical laboratories, with concentration and/or enrichment procedures incorporated before the organisms are transferred to harsh selective media.

Although the role and relevance of follow-up laboratory investigations of waterborne outbreaks have been questioned, the cumulative knowledge obtained from these investigations may lead to new preventative technical measures and may, therefore, be of great value in preventing future outbreaks (3). Furthermore, investigations of these outbreaks have led to the identification of waterborne pathogens not previously considered to be important, such as *Y. enterocolitica* and *C. jejuni*, as well as pathogens not previously recognized. *L. pneumophila* was not recognized until after the follow-up investigation of a 1976 pneumonia outbreak (4).

Isolation and Enumeration of Enteric Pathogens

Salmonella

Good hygiene and modern sewage and potable water treatment practices have dramatically reduced the incidence of illness caused by *Salmonella*. Even though the number of outbreaks has declined since the mid-20th century, *Salmonella* must still be considered a threat to public health. The waterborne outbreak of salmonellosis that occurred in Riverside, California, in 1965 verified the continued significance of this organism. Over 16,000 cases of illness resulted from that single outbreak (5).

Because of the low densities at which *Salmonella* occurs in water, concentration and enrichment procedures are normally used for isolating this organism. Usually water samples suspected to contain *Salmonella* are concentrated by filtration either through membranes, if the samples are relatively clear, or through diatomaceous earth if the samples are highly turbid (6,7). Alternatively, absorbent pads suspended in the water for long periods of time (8) or filtration through fiberglass epoxy depth filters (9) may be used.

After concentration, the membranes or filter materials are incubated in an enrichment medium to increase the number of salmonellae relative to coliforms and enhance the probability of isolating *Salmonella*. Quantitative estimates of the number of salmonellae in drinking water or other waters containing low densities of pathogens can be obtained by using filtration and subsequent enrichment in conjunction with most probable number (MPN) estimates. With these samples, three sets of decimal volumes are substituted for decimal dilutions (9,10). The enrichment media recommended for water samples are dulcitol-selenite broth or tetrathionate broth. The advantages of using dulcitol-selenite broth are that it allows the rapid growth of *Salmonella* while inhibiting many nonpathogenic enterobacteria and that it causes cultures containing salmonellae to develop a distinct orange or red color. The advantage of tetrathionate broth is that it may yield greater numbers of salmonellae (7).

Primary enrichment is followed by secondary differentiation on selective solid media, such as brilliant green agar (BG), bismuth sulfite agar (BS), or xylose lysine deoxycholate agar (XLD). *Salmonella* colonies appear pinkish-white with a red background on BG, black (with or without a metallic sheen) on BS, and red with black centers on XLD. Use of an incubation temperature of 41.5°C enhances the isolation of many salmonellae (8). However, some serotypes, including *S. typhi,* do not grow at this elevated temperature. When *S. typhi* is suspected as the cause of an outbreak, BS agar and a growth temperature of either 35 or 37°C should be used (6).

Because other enteric organisms of little or no pathogenicity share some major biochemical characteristics with *Salmonella*, it is necessary to confirm the identify of suspect colonies by biochemical tests or serotyping. Serotyping is a particularly valuable technique because it is highly specific. The isolation of a *Salmonella* from water with the same serotype as that of patients' isolate confirms an outbreak as waterborne.

Shigella

The shigellae are among the most common causes of diarrhea in humans. All four species of the genus *Shigella* (*S. dysenteriae, S. flexneri, S. boydii,* and *S. sonnei*) are pathogenic (11), and these pathogens have been implicated in a number of waterborne outbreaks (12). Because the shigellae are highly specific for humans, human cases are always the source of infection (11).

The procedures used to isolate *Shigella* sp. and other enteric pathogens from water are fundamentally the same as those used to isolate the salmonellae: concentration by filtration, selective enrichment, isolation on selective differential plating media, and confirmation by biochemical and serological techniques.

Water samples suspected to contain shigellae are filtered as described in the section on *Salmonella*. The filters or filter materials are either placed in Gram-negative (GN) broth for nonselective enrichment (13-15) or in the more effective autocytotoxic medium developed by Park and colleagues (16,17). The autocytotoxic medium is a lactose broth containing 4-chloro-2-cyclopentyl-phenyl-ß-D-galactopyranoside, an analogue of lactose. Coliforms and other organisms that utilize lactose hydrolyze this compound to galactose and a toxic moiety that subsequently kills them. Shigellae, which do not utilize lactose, are not affected. The improved formula for this medium allowed selective enrichment of 42 of 48 *Shigella* strains from all four species in competition with a 1,000-fold higher population of *Escherichia coli* (17). This medium can be used in a modified MPN procedure to obtain a quantitative estimate of the number of shigellae in water samples.

The results of several studies indicate that XLD agar is the best selective differential plating medium for isolating *Shigella* from enrichment broths (13,15,18). Colonies of *Shigella* are red and can easily be distinguished from *Salmonella* colonies, which are red with black centers. Although the shigellae are very closely related to *E. coli*, they can be differentiated from *E. coli* either by a battery of 13 biochemical tests or by serology (19).

Yersinia Enterocolitica

Y. enterocolitica is a relatively new addition to the list of bacterial pathogens known to cause waterborne gastroenteritis. The first report of waterborne illness attributed to *Y. enterocolitica* was in 1972 (20). The requirements of *Y. enterocolitica* for special growth conditions compared to other *Enterobacteriaceae* was no doubt partly responsible for the failure to recognize this pathogen earlier (21). The most common manifestation of infection is a diarrheal disease with abdominal pain and fever. The occurrence of abdominal pain in the lower right quadrant has sometimes confused the diagnosis as appendicitis (21).

One of the characteristics of *Y. enterocolitica* that is unique among the *Enterobacteriaceae* is the ability to grow well in suitable media at refrigeration temperatures (21). The traditional method for enrichment of *Y. enterocolitica* from fecal specimens, incubation at 4°C for 3 weeks, took advantage of this characteristic and allowed *Y. enterocolitica* to grow to higher densities than the other enteric flora (22). *Y. enterocolitica* has been isolated from contaminated water after only 9 days incubation at 4°C (23). However, this is still an inconveniently long incubation time. Weagant and Kaysner (24) developed a 22°C enrichment procedure that required incubation for only 48 hours. Their enrichment medium may be inoculated directly, or samples may be filtered and filters placed into the medium. Sorbitol is the primary differential substrate in this medium.

A comparative study by Head *et al.* (25) identified the Cefsulodin-irgasan- novobiocin (CIN) medium developed by Schiemann (26) as the most efficient selective differential agar medium for isolating *Y. enterocolitica*. This medium uses antibiotics and bile salts for suppression of competing bacterial flora and a differential reaction resulting from mannitol fermentation for discrimination of *Y. enterocolitica* from most other Gram-negative bacteria able to grow on the medium. *Y. enterocolitica* colonies on CIN agar have a deep red center surrounded by an outer zone that is usually translucent. Although incubation at 32°C for 24 hours may be used, mannitol fermentation is stronger and more complete with incubation at 22°C for 48 hours. Further confirmation may be obtained by inoculation on lysine-arginine-iron agar (LAIA) slants (27). Typical reactions of *Yersinia* sp. on LAIA are alkaline slant (purple), acid butt (yellow), no H_2S (darkening of butt), or gas formation. Biotyping and serotyping procedures are also available (28,29) as is a recently developed DNA probe that identifies virulent *Y. enterocolitica* colonies (30,31).

A membrane filter (MF) method is also available for enumerating and isolating *Y. enterocolitica* from large volumes of low-turbidity water (32). This method is particularly advantageous because it allows for presumptively identifying the organism without transferring colonies to multiple confirmatory media. A sample is filtered through a membrane filter, and the filter is placed on a cellulose pad saturated with a recovery broth (mYE medium). After incubation for 48 hours at 25°C, the membrane is asceptically transferred to a lysine-arginine agar substrate and incubated anaerobically at 35°C for 1 hour. A needle is then used to puncture a hole in the membrane next to each yellow to yellow-orange colony, and the membrane is transferred to a urease-saturated absorbent pad and incubated at 25°C for 5 to 10 min. All marked distinctly green or bluish colonies are counted immediately. These colonies are sorbitol-positive, lysine- and arginine-negative, and urease-positive; and they may be presumptively identified as *Y. enterocolitica*.

Pathogenic Escherichia Coli

There are five categories of *Escherichia coli* responsible for a wide diversity of gastrointestinal illnesses: enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), and enteroadherent (EAEC). The ETEC are a major cause of travelers' diarrhea and infant diarrhea in less-developed countries. These strains produce heat-labile enterotoxin (LT), heat-stable enterotoxin (ST), or both. The EIEC are a cause of dysentery and are distinguished from other pathogenic *E. coli* by their capacity to invade and proliferate within epithelial cells. The EPEC are an important cause of infant diarrhea. EPEC strains are pathogens but cause diarrhea by some mechanism distinct from LT, ST, or *Shigella-like* invasiveness. The EHEC, a cause of hemorrhagic colitis and hemolytic uremic syndrome, elaborate phage-encoded cytotoxins active on Vero and HeLa cells (VTs). Most EHEC have the 0157:H7 serotype. The EAEC are a less well-defined category of diarrheagenic *E. coli* that are identifiable only by their pattern of adherence to Hep-2 cells. Within each of these categories the strains tend to fall within certain O:H serotypes (33).

Although it seems unlikely that *E. coli* could be transmitted through treated potable water, that possibility does exist. Potentially pathogenic *E. coli* have been isolated from tap water (34), and a waterborne outbreak of gastroenteritis affecting 251 passengers and 51 crew members on a crew ship was attributed to ETEC (35).

Detection of *E. coli* in water implicated in an outbreak may be achieved either by the MPN or MF techniques described in a later section (Indicator Organisms). Confirmation, however, depends on either matching the serotype of the water isolate with that of the clinical isolate or on verifying the pathogenicity of the isolate. Traditional tests used to differentiate pathogenic *E. coli* include suckling mouse and rabbit ileal loop bioassays and Y-1 adrenal cell and Chinese hamster ovary cell assays for ETEC and the Sereney test for EIEC (36). One procedure for the identification of ETEC that produce LT was developed specifically for isolating and enumerating these organisms from water. ETEC colonies on a modified MF medium were identified by transferring the membrane to Y-1 adrenal cell monolayers; colonies producing LT in approximately 2 days caused the Y-1 cells to round up (37).

Advances in biotechnology have led to more rapid and facile techniques that may soon make all of the above assays obsolete. Enzyme linked immunosorbent assay (ELISA) techniques are available for identifying LT-, ST-, and VT-producing strains (38-41); and DNA probes are available for identifying ETEC that produce either LT or ST, EIEC, EPEC, and EHEC (42-47). DNA probe methodology includes both dot-blot (43) and colony hybridization (42,44,47) methods.

Campylobacter jejuni

Campylobacter jejuni is another recent addition to the growing list of known enteric pathogens. This organism was not generally recognized as a common cause of diarrhea until 1977 (48). The fairly uncommon conditions for optimal growth of *C. jejuni*, i.e., microaerophilic atmosphere and 42°C, incubation apparently contributed to the late recognition of this pathogen (49).

C. jejuni and closely related *C. coli* cause a gastroenteritis with bloody diarrhea and abdominal cramps (50,51). Numerous animal species have been cited as reservoirs of campylobacters (52). The most common sources of human infection are unpasteurized milk, chicken, and water (49). Although campylobacters are sensitive to chlorine, municipal water systems have been the sources of outbreaks, presumably due to breakdowns in treatment or contamination after chlorination (1,53-55).

Methods originally developed for isolation of campylobacters from fecal specimens have been successfully used to isolate these organisms from water (55-57). The methods involve filtration of several liters of the water sample, followed either by enrichment in a broth medium or placing the filter on a selective agar medium, with subsequent incubation under microaerophilic conditions at 42°C. Pressure filtration in a stainless steel filtration device with a 1.5 L reservoir may be necessary for samples that are turbid (7).

Methods for isolating campylobacters from environmental samples are not standardized, and there is still no specific method recommended above all others. Several MPN methods have been used.

The traditional medium, "Campythio broth," which was developed in 1979 by Blaser *et al.* (58), is still sometimes used in environmental isolations (59). Another medium was developed by Oosterom *et al.* (60) for detecting *Campylobacter* in sewage and river samples. However, there is very little supporting information concerning the use of that medium. Bolton and Robertson (61) developed a selective medium which they called Preston medium. The Preston medium without agar was used as an enrichment broth. Preston agar was compared with Skirrow's (48) agar for recovery of *Campylobacter* from the feces of humans and several other species. The results showed that the Preston agar recovered more *Campylobacter* than the Skirrow agar, and if the Preston enrichment broth was also used, considerably more *Campylobacter* isolates were found. These investigators reported successfully using the combination of Preston enrichment broth and Preston agar to isolate campylobacters from coastal seawater samples, drain swab effluent, and environmental specimens from local abattoirs. Bolton *et al.* (62) reported that the use of Preston enrichment broth followed by subculture on Preston agar allowed the estimation of as few as 10 campylobacters per 100 mL of water. Mathewson *et al.* (63) evaluated several types of filters and determined that a positive-charged depth filter gave the highest percent recovery of campylobacters from seeded tap and surface waters. This type of filter should enhance the sensitivity of the filter enrichment MPN technique.

Several selective plating media, in addition to Preston medium, are available for isolating *Campylobacter* from enrichment broth. These include Skirrow's medium (48), Campy blood agar (58), Butzler's medium (64), and a blood-free medium developed by Bolton *et al.* (65). On these media, *Campylobacter* forms spreading colonies that range in color from clear to white or tan. Suspect colonies should be verified biochemically (49,66). Hutchinson *et al.* (67) and Dilworth *et al.* (68) recommended further confirmation using a combination of serotyping and biotyping.

Several different serotyping systems have been developed and published, creating a very complicated situation. It was recommended at the Third International Workshop on *Campylobacter* infections in Ottawa, Ontario, in 1985 that only two of these systems should be used. One is based on the heat-labile antigens using slide agglutination as described by Lior *et al.* (69) and the other is based on heat-stable antigen using passive hemagglutination as described by Penner *et al.* (70). Biotyping schemes have also been developed by Lior (71) and Bolton *et al.* (72).

Campylobacter sp. may also be directly isolated by the MF procedure using any of the selective media described above. Rosef *et al.* (73) reported that enrichment did not significantly enhance the recovery of thermotolerant campylobacters from naturally contaminated water samples over that produced by direct plating of the membrane filters onto Preston agar or colistin-amphotericin-keflin (CAK) agar. However, several other researchers have reported that the MT procedure was less effective than selective enrichment procedures. Bolton *et al.* (62) reported that the MF procedure was unsuitable for the enumeration of campylobacters because of the tendency of colonies developing on the membrane to spread and coalesce. Ribeiro *et al.* (74) reported that 12 out of 64 water samples that were *Campylobacter*-negative by direct plating were positive if the filters were placed in Preston enrichment broth and subsequently subcultured onto a selective medium. Bolton *et al.* (75) compared Skirrow's, Butyle's, Blaser's, Campy-BAP, and Preston medium for efficiency in isolating *Campylobacter* from fecal and environmental specimens. They found that Preston agar, which was the most selective, gave the highest isolation rate and that preenrichment in Preston broth gave a higher isolation rate than direct plating on Preston agar. Goosens *et al.* (76) found that the MF method was easy and cheap, but low in sensitivity. The low sensitivity of the MF method reported by Goosens *et al.* (76) may be partially due to their use of 0.45 µm filters. Researchers from the U.S. Food and Drug Administration report that recovery is significantly enhanced if 0.22 µm filters are used (J.M. Hunt, personal communication).

Isolation of campylobacters from source waters in the absence of an outbreak should be interpreted with caution. *Campylobacter* organisms that closely resembled *C. jejuni* but were apparently nonpathogenic were isolated from a variety of water samples by Mawer (77,78). These atypical strains can be distinguished from *C. jejuni* by their inability to hydrolyze hippurate.

Indicator Organisms

Total Coliforms

Sometimes the pathogenic bacterium that caused a waterborne outbreak will have died off or reached too low a density to be recovered by the time the water is sampled and analyzed. When this situation occurs, isolation of an indicator of fecal contamination from epidemiologically implicated water can provide further evidence that the outbreak was waterborne. Indicators are normally present in fecally contaminated waters in higher numbers than pathogens and consequently persist after the pathogen can no longer be isolated.

The coliforms are defined as the group of bacteria that are Gram-negative, aerobic or facultatively anaerobic, nonsporeforming rods that ferment lactose with the production of gas within 48 hours at 35°C (7). This group of organisms was originally believed to indicate the presence of fecal pollution, and they are still used for that purpose (2). The finding of coliforms in source waters or drinking water is interpreted as either a degradation of the quality of water or ineffective treatment, even though some coliforms are widely distributed in nature and are not associated with the intestinal tract of warm-blooded animals (79).

The methods approved by the U.S. Environmental Protection Agency (U.S. EPA) and recommended in Standard Methods for the Examination of Water and Wastewater (7) are the lauryl sulfate tryptose broth (LTB) MPN method and the MF procedure employing m- Endo or m-Endo LES agar.

The MPN procedure uses five fermentation tubes for each of three decimal multiples of 1 mL. Formation of gas in the inner fermentation tubes constitutes a presumptive positive test. Presumptive positive cultures must be verified by growth and gas production in brilliant green lactose broth. A completed test requires transfer onto m-Endo LES or eosin methylene blue (EMB) agar slants and Gram-staining (7).

A new method called the "Autoanalysis Colilert" (80) apparently does not require confirmatory or completed tests. To perform this test, one only has to add the water sample to the powdered ingredients in a tube or flask and incubate for 24 hours. If total coliforms are present in the water sample, the solution will change from its colorless state to yellow. This test can be used as a MPN test and will detect 1 total coliform per 100 mL in a maximum of 24 hours.

The standard MF procedure utilizes m-Endo broth or agar. Organisms that produce a colony with a golden-green metallic sheen on this medium within 24 hours of incubation are considered presumptive members of the coliform group. Coliform organisms may occasionally produce atypical colonies. The identity of these colonies as coliforms can be verified by inoculating them into LTB and brilliant green lactose bile broth and observing the tubes for gas formation within 48 hours of incubation at 35 + 0.5°C (7). To save time, both media may be inoculated simultaneously.

Observation of many false-positive and false-negative results and poor recovery of stressed cells on m-Endo agar (81,82) led to the development of m-T7 medium by LeChevallier *et al.* (83). They reported recovering an average of 43% more verified coliforms from surface and drinking water samples than were recovered on m-Endo medium, due to the recovery of higher numbers of injured coliforms. Coliform organisms produce smooth, yellow, convex colonies on M-T7. LeChevallier *et al.* (84) also proposed a new verification test for coliforms from MF methods to substitute for the subculturing of colonies to lactose broth and brilliant green bile broth. This method, which utilizes testing for the presence of β-galactosidase and absence of cytochrome oxidase, increases the confirmation rate by 87 percent over the currently accepted methods.

Another new MF medium (m-HARC) developed at the U.S. EPA Cincinnati, Ohio, laboratories has excellent potential. Although this medium does not recover significantly more coliforms than are recovered on m-Endo, it has the advantages of allowing coliforms to be counted more easily and of not requiring proprietary ingredients. Coliform colonies on m-HARC are yellow against a cobalt blue background, whereas noncoliform colonies are blue or green (J.R. Haines, personal communication).

Fecal Coliforms

Fecal coliforms are a subgroup of the total coliforms that can be differentiated by their ability to grow at 44.5 + 0.2°C (7). This thermotolerant characteristic usually separates coliforms that may be found in the GI tract of warm-blooded animals from the other coliforms. Therefore, a positive test for fecal coliforms is a more specific indication of fecal contamination than a positive test for total coliforms. The fecal coliform test is not recommended for routine examination of potable waters, because no coliform of any kind should be tolerated in treated water (7). However, this test may be quite useful in an outbreak investigation to aid in establishing the vehicle of the outbreak.

This group of organisms is identified either by the MPN procedure using EC broth and fermentation tubes or the MF method using m-FC medium. Gas production in a fermentation tube within 24 hours or less is considered a positive MPN reaction, indicating coliforms of fecal origin (7). A positive test on m-FC medium is indicated by the appearance of dark blue colonies; all other colonies are light blue to gray to cream colored (85). Few nonfecal coliform colonies will be observed on m-FC medium because of the selective action of the elevated temperature and the presence of a rosolic acid salt reagent (7).

Stuart *et al.* (86) compared a two-layer MF medium (IM-MF) for injured fecal coliforms with the EC broth MPN procedure and the standard m-FC method using various types of water samples. They observed that recoveries by the IM-MF method were equal to or greater than those by the MPN method in 50% of the samples tested and greater than the m-FC method in all of the samples.

Escherichia coli

A positive test for *E. coli* in a water sample is a more definite indication of fecal contamination than is a positive fecal coliform test. *E. coli*, one of the members of the fecal coliform group, is an unquestionable inhabitant of the GI tract of humans and other warm-blooded animals, whereas some of the other so-called fecal coliforms are of environmental origin. *E. coli* is easily distinguished from the other members of the fecal coliform group by the urease test. *E. coli* does not possess this enzyme, whereas the other members of the coliform group do. Dufour *et al.* (87) used this characteristic to develop a MF method that was 90 percent specific for *E. coli* from marine, estuarine, and fresh water supplies. The method incorporates a lactose-base selective differential medium (mTEC), a 2-hour resuscitation of weakened organisms at 35°C before incubation at 44.5°C for 18 to 22 hours and an *in situ* test for urease. Yellow colonies obtained after the urease test are presumed to be *E. coli*.

The developers of the new "Autoanalysis Colilert" test for total coliforms contend that the method can also be used as a MPN method for *E. coli*. The medium contains 4-methylumbelliferyl-β-D-glucuronide (MUG), which is hydrolyzed by *E. coli* but not by other members of the coliform group. One of the metabolites of MUG fluoresces when exposed to ultraviolet light. Species in other genera, such as *Aeromonas*, *Pseudomonas*, and *Flavobacterium*, that could be positive in a MUG assay do not grow in the restrictive medium and will not be positive in this growth-based test unless a high density (≥20,000 bacteria per mL) is present (80).

Opportunistic Pathogens

Legionella Species

Legionella pneumophila is a Gram-negative pleomorphic bacterium that was identified in 1977 as the causative agent of the pneumonic illness, Legionellosis (Legionnaires' disease) (88). *L. pneumophila* and related organisms have been assigned to the family *Legionellaceae*, which consists of a single genus (*Legionella*) and a number of species (89). The recently described *L. cincinnatiensis* is the 25th species and the 41st serogroup described in the genus and the 14th species shown to be pathogenic for humans (90). Epidemiological data suggest that about two thirds of reported cases occur among individuals who smoke and/or have significant underlying conditions (91).

The *Legionellaceae* are ubiquitous in aquatic environments, including potable water (92-94). Even water taps, water storage tanks, and showerheads may provide suitable environments for growth of legionellae (95). The usual mechanism of transmission appears to be aerosolization of water containing legionellae (96).

Legionella is also the etiologic agent for a second type of illness called Pontiac fever. This illness is characterized by a short (1- to 2-day) incubation period, a self-limited grippelike illness, without pneumonia, and a high attack rate (100% in one outbreak) (97-99).

Three media are available for isolating legionellae. All three use the charcoal, yeast extract, cysteine, ferric pyrophosphate agar base developed by Feeley *et al.* (100). Bopp *et al.* (101), Wadowsky and Yee (102), and Edelstein (103) modified this basal medium by adding various combinations of antibiotic inhibitors. Calderon and Dufour (104) compared the efficiencies of these media for eliminating background flora and recovering *Legionella*. They found that the modification developed by Edelstein was the most efficient of the three media for recovering legionellae seeded into environmental samples. Recommended incubation conditions are 35°C and 90 percent relative humidity if the buffered medium developed by Edelstein is used, and the above conditions plus 2.5 percent CO_2 if unbuffered media are used (103). *Legionella* colonies should be visible on all of the charcoal, yeast extract-based media in 3 to 5 days, appearing light blue and having a cut-glass texture. On continued incubation, colonies will become whiter, smoother and larger in diameter (105).

Several cultural and biochemical characteristics are helpful in distinguishing legionellae from other bacteria (105). Gram-negative organisms that resemble L. *pneumophila* in colonial morphology and grow only on L-cysteine-HCl-containing media are probably legionellae, whereas organisms that grow on ordinary blood agar are definitely not. Although the legionellae are positive for enzymes such as catalase and oxidase, they give negative reactions for most biochemical tests; and an isolate can only be definitively identified by results from highly specific methods such as the direct fluorescent-antibody test (DFA) (106), DNA probes (107,108), or monoclonal antibody techniques (109,110).

If rapid presumptive identification is required, sedimented material from environmental samples can be examined directly by DFA. However, the DFA procedure gives some cross-reactivity with other species (111) and does not distinguish between viable and nonviable organisms. Therefore, the presence of *Legionella* in an environmental sample should be confirmed using direct isolation procedures (7).

Barbaree *et al.* (112) developed a detailed protocol for sampling environmental sites for legionellae. Their protocol included recommended numbers of samples and sample volumes for a variety of sites, including water treatment plants, different sites within hospital water systems, air-conditioning systems and whirlpools. They recommended inoculating duplicate plates of each of two kinds of media with 0.1 mL of each concentrated or nonconcentrated sample. The recommended media were buffered charcoal-yeast extract agar containing alpha-keloglutarate (αBCYE) and BCYE supplemented with glycine, polymyxin B, anisomycin and vancomycin (GPAV). They also suggested inoculating one plate of αBCYE and one plate of GPAV without L-cysteine as controls.

It may be advantageous to add a heat-shock step to the isolation procedure when cold waters are examined A recent study by Hussong *et al.* (113) provided evidence that *L. pneumophila* sometimes exists in a viable but not culturable state. Colbourne *et al.* (114) discovered that culturability was related to temperature above 20°C and that heat-treatment of culture-negative distribution water samples at 45°C for either 10 or 20 min allowed them to recover viable *L. pneumophila*.

There are several methods that have been used to correlate environmental isolates of *Legionella* with those from patients. Serogrouping has typically been used as an epidemiological marker. However, additional markers such as plasmids (115) and panels of monoclonal antibodies (109,110) are useful when the same serotype is isolated from two or more discrete environmental points.

Mycobacterium Sp.

Several of the nontuberculosis mycobacteria have been associated with opportunistic infections. Species implicated in this type of infection include *M. kansasii, M. xenopi, M avium*, the intracellular variant of *M. avium*. and *M. scrofulaceum* (116). Two of these species or complexes *M kansasii* and *M. avium* complex (MAC), are the prominent cause of disseminating mycobacteriosis in the United States (117). The rate of occurrence of MAC has increased markedly in recent years, primarily because the epidemic of acquired immunodeficiency syndrome (AIDS) has caused an increase in the number of susceptible individuals (118). Data collected in Massachusetts showed a five-fold increase in the incidence of isolation of MAC from clinical specimens between 1972 and 1983 (119).

The etiology and pathophysiology of mycobacteriosis has not been fully elucidated. However, water has been suspected for many years as a source of potentially pathogenic mycobacteria (116,120). Aerosolization may be a mechanism for transmission. These organisms are relatively resistant to chlorine (121); and have been isolated from chlorinated sources, including swimming pools, taps, drinking fountains, and aquariums (120). Du Moulin et al (122) found that mycobacteria survive and proliferate in water with temperature ranges of 52 to 57°C and that potable hot water systems, particularly those that generate aerosols, may contain concentrations of *M. avium* greater than those found in cold water. They speculated that these systems could serve as environmental sources for colonization and infection of immunocompromised persons.

The first step in isolating mycobacteria from water is concentrating the sample either by centrifugation or filtration. The next step is decontamination to eliminate unwanted competing bacteria. For this, several decontamination procedures have been used. Initially either sodium hydroxide (123-125) or a mixture of sodium hydroxide and sodium hypochlorite (120) was used. Later, du Moulin and Stottmeier (126) developed a method more suitable for large volume water samples. Their method substitutes overnight exposure to 0.4% cetylpyridinium chloride for alkali treatment. This method eliminates the adverse effects of the alkali on membrane filters and the suppressive effect of alkali on the growth of mycobacteria.

The final step of the isolation procedure involves placing the concentrated sample or the membrane filter on a selective medium.

Middlebrook 7H10 is the basal medium most frequently used. Studies have employed several modifications of this medium, with various combinations of antibiotics added to the basal medium. Petran and Vera (127) added cyclohexamide, lincomycin, and nalidixic acid to inhibit Gram-negative competitors. Lorain and Maddock (128) incorporated colimycin, spiramycin, and amphotercin B. Mitchison et al (129) developed a 7H11 medium that contained polymyxin B, carbenicillin, amphotercin B, and trimethoprim. Du Moulin *et al.* (122) reported that adjusting the pH of Middlebrook Cohn 7H10 to 5.5 gave optimal recovery of waterborne mycobacteria.

Growth of mycobacteria is slow on all laboratory media. *M. fortuitum* and *M. chelonii* may form colonies in <1 week. However, most strains of *Mycobacerium* require 2 or more weeks to form visible colonies (130).

Species identification and confirmation may be accomplished by a combination of traditional methods such as colony morphology (130), biochemical tests (120,130), antimicrobial susceptibility tests (118, 131), and seroagglutination (118); or by more sophisticated techniques such as monoclonal antibodies (132), DNA probes (133,134), or analysis of mycolic acids by high-performance liquid chromatography (HPLC) (135).

Summary

Two types of pathogens have been implicated in illness outbreaks attributed to potable water. The first type consists of pathogens of fecal origin, including *Salmonella, Shigella,* and *Campylobacter*. These pathogens are normally inactivated by water treatment processes but may get into distribution systems through temporary breakdowns in treatment or through post-treatment contamination with sewage. Isolation of these pathogens from potable water is difficult because of their low numbers and transient existence in drinking water and because of competition by numerous organisms with less fastidious nutrient requirements. These difficulties are usually minimized by using selective enrichment procedures prior to isolation on selective differential agar media.

The isolation procedures for all of the fecal pathogens are fundamentally the same: concentration by filtration, selective enrichment in broth media, isolation on selective differential agar media, and verification of the species by biochemical and serological tests. In some cases, DNA probes can now be used for verification.

In some outbreaks, the suspected pathogen may no longer be detectable when the water is analyzed. When this occurs, isolation of organisms that are indicators of fecal contamination can provide evidence that epidemiologically implicated drinking water was the vehicle for infection. The presence of total coliforms, the traditional indicator used for measuring degradation of potable water quality, can be measured either by MPN or MF techniques. Similar techniques are available for estimating the numbers of fecal coliforms, a subgroup of total coliforms more likely to be of fecal origin, and for *E. coli*, an organism of undisputed fecal origin.

Other pathogens implicated as causes of waterborne illness are opportunistic pathogens that are capable of long-term survival and growth within potable water distribution systems. Known pathogens of this type include many of the legionellae and nontuberculosis mycobacteria. These organisms are resistant to chlorine and are capable of growth at high water temperatures. They are probably disseminated by aerosols. Isolation and identification of these pathogens are difficult due to their slow growth rates, their requirements for highly specialized growth media, and the negative reactions they give in most biochemical tests. Sophisticated rapid methods, such as DNA probes, panels of monoclonal antibodies, and HPLC, are now available as alternatives to traditional biochemical tests.

References

1. Craun, G.F. Recent statistics of waterborne disease outbreaks (1981-1983). *In:* G.F. Craun (ed.), Waterborne Diseases in the United States, CRC Press Inc., Boca Raton, Florida, 1986.
2. Ericksen, T.N. and Dufour, A.P. Methods to identify waterborne pathogens and indicator organisms. *In:* G. F. Craun (ed.), Waterborne Diseases in the United States, CRC Press, Inc., Boca Raton, Florida, 1986.
3. Anderson, Y. and Senstrom, T.A. Waterborne outbreaks in Sweden – causes and etiology. *Wat. Sci. Tech.* 19:575, 1987.
4. McDade, J.E., Shepard, C.C., Fraser, D.W., Tsai, T.R., Redus, M.A., and Dowdle, W.R. Legionnaires disease: isolation of a bacterium and demonstration of its role in other respiratory disease. *New England. J. Med.* 297:1197, 1977.
5. A waterborne outbreak of salmonellosis in Riverside, California, 1965: epidemiological aspects. *Am. J. Epidemiol.* 93:33, 1971.
6. Cheng, C.M., Boyle, W.C. and Goepfert, J.M. Rapid quantitative method for *Salmonella* detection in polluted waters. *Appl. Microbiol.* 21:662, 1971.
7. Standard Methods for the Examination of Water and Wastewater, 16th ed. American Public Health Association, Washington, D.C., 1985.
8. Spino, D.F. Elevated temperature technique for the isolation of *Salmonella* from streams. *Appl. Microbiol.* 14:591, 1966.

9. Levin, M.A., Fischer, J.R., and Cabelli, V.J. Quantitative large-volume sampling technique. *Appl. Microbiol.* 28:515, 1974.

10. Grunnet, K. Quantitative *Salmonella* demonstration by pad technique. *Rev. Inter. Oceanogr. Med.* 34:155, 1974.

11. Orskov, F. and Orskov, I. Enterobacteriaceae *In:* A.I. Braude, C.E. Davis, and J. Fierer (ed.), Medical Microbiology and Infectious Diseases. W.B. Saunders Co., Philadelphia, Pennsylvania, 1981.

12. Craun, G.F. Statistics of waterborne outbreaks in the U.S. (1920-1980). *In:* G.F. Craun (ed.), Waterborne Diseases in the United States. CRC Press, Inc., Boca Raton, Florida, 1986.

13. Dunn, C. and Martin, W.T. Comparison of media for isolation of salmonellae and shigellae from fecal specimens. *Appl. Microbiol.* 22: 17, 1971.

14. Taylor, W.I. and Schelhart, D. Isolation of shigellae. VIII. Comparison of xylose lysine deoxycholate agar, hektoen enteric agar, *Salmonella-Shigella* agar and eosin methylene blue agar with stool specimens. *Appl. Microbiol.* 21:32, 1971.

15. Taylor, W.I. and Schelhart, D. Effect of temperature on transport and plating media for enteric pathogens. *J. Clin. Microbiol.* 2:281, 1975.

16. Park, C.E., Rayman, M.K., Szabo, R. and Stankiewicz, Z.K. Selective enrichment of *Shigella* in the presence of *Escherichia coli* by the use of 4-Chloro-2-Cyclopentylphenyl-β-D-galactopyranoside. *Can. J. Microbiol.* 22:654, 1976.

17. Park, C.E., Rayman, M.K., and Stankiewicz, Z.K. Improved procedure for selective enrichment of *Shigella* in the presence of *Escherichia coli* by use of 4-Chloro-2-Cyclopentylphenyl-β-D-galacctopyranoside. *Can. J. Microbiol.* 23:563, 1977.

18. Taylor, W.I. and Harris, B. Isolation of shigellae. II. Comparison of plating media and enrichment broths. *Amer. J. Clin. Pathol.* 44:476, 1965.

19. Martin, W.J. and Washington, J.A. II. Enterobacteriaceae. *In:* E.H. Lennette, Balows, A., Hausler, W.J. and Truant, J.P. (eds.), Manual of Clinical Microbiology, 3rd ed. American Society for Microbiology, Washington, D.C., 1980.

20. Lassen, J. *Yersinia enterocolitica* in drinking water. *Scand. J. Infect. Dis.* 4:125, 1972.

21. Schiemann, D.A. A synoptic review on *Yersinia enterocolitica*. *J. Environ. Health.* 44:183, 1982.

22. Highsmith, A.K., Feeley, J.C. and Morris, G.K. *Yersinia enterocolitica:* a review of the bacterium and recommended laboratory methodology. *Health Lab. Sci.* 14:253, 1979.

23. Thompson, J.S. and Gravel, M.J. Family outbreak of gastroenteritis due to *Yersinia enterocolitica* serotype 0:3 from well water. *Can. J. Microbiol.* 32:700, 1986.

24. Weagant, S.D. and Kaysner, C.A. Modified enrichment broth for isolation of *Yersinia enterocolitica* from nonfood sources. *Appl. Environ. Microbiol.* 45:468, 1983.

25. Head, C.B., Whitty, D.A. and Ratnam, S. Comparative study of selective media for recovery of *Yersinia enterocolitica. J. Clin. Microbiol.* 16: 615, 1982.

26. Schiemann, D.A. Synthesis of a selective agar medium for *Yersinia enterocolitica. Can. J. Microbiol.* 25:1298, 1979.

27. Weagant, S.D. Medium for presumptive identification of *Yersinia enterocolitica. Appl. Environ. Microbiol.* 45:472, 1983.

28. Sonnenwirth, A.C. *Yersinia. In:* E.H. Lennette, Spaulding, E.H. and Truant, J.P., (eds.), Manual of Clinical Microbiology, 2nd ed. American Society for Microbiology, Washington, D.C., 1974.

29. Swaminathan, B., Harmon, M.C. and Mehlman, I.J. A review: *Yersinia enterocolitica. J. Appl. Bacteriol.* 52:151, 1982.

30. Jagow, J. and Hill, W.E. Enumeration by DNA colony hybridization of virulent *Yersinia enterocolitica* colonies in artificially contaminated food. *Appl. Environ. Microbiol.* 51:441, 1986.

31. Jagow, J. and Hill, W.E. Enumeration of virulent *Yersinia enterocolitica* colonies by DNA colony hybridization using alkaline treatment and paper filters. *Molec. Cell. Probes.* 2:189, 1988.

32. Bartley, T.D., Quan, T.J., Collins, M.T., and Morrison, S.M. Membrane filter technique for the isolation of *Yersinia enterocolitica. Appl. Environ. Microbiol.* 43:829, 1982.

33. Levine, M.M. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. *J. Infect. Dis.* 155:377, 1987.

34. Ewing, W.H. Sources of *Escherichia coli* cultures that belong to 0-antigen groups associated with infantile diarrheal disease. *J. Infect. Dis.* 110:114, 1962.

35. O'Mahony, M.C., Noah, N.D., Evans, B., Harper, D., Rowe, B., Lowes, J.A., Pearson, A., and Goode, B. An outbreak of

gastroenteritis on a passenger cruise ship. *J. Hyg. Camb.* 97:229, 1986.

36. Sack, R.B. Enterotoxigenic *Escherichia coli:* identification and characterization. *J. Infect. Dis.* 142:279, 1980.

37. Calderon, R.L. and Levin, M.A. Quantitative method for enumeration of enterotoxigenic *Escherichia coli*. *J. Clin. Microbiol.* 13:130, 1981.

38. Sack, D.A., Huda, S., Neogi, P.K.B., Daniel, R.R., and Spira, W.M. Microtiter ganglioside enzyme-linked immunosorbent assay for *Vibrio* and *Escherichia coli* heat-labile enterotoxins and antitoxin. *J. Clin. Microbiol.* 11:35, 1980.

39. Thompson, M.R., Brandwein, H., Labine-Racke, M., and Gianella, R.A. Simple and reliable enzyme-linked immunosorbent assay with monoclonal antibodies for detection of *Escherichia coli* heat-stable enterotoxins. *J. Clin. Microbiol.* 20:59, 1984.

40. Karch, H., Strockbine, N.A., and 0'Brien, A.D. Growth of *Escherichia coli* in the presence of trimethoprim-sulfamethoxazole facilitates detection of shiga-like toxin-producing strains by colony blot assay. *FEMS Microbiol. Lett.* 35:141, 1986.

41. Kongmuang, U., Honda, T., and Miwatani, T. Enzyme-linked immunosorbent assay to detect shiga toxin of *Shigella dysenteriae* and related toxins. *J. Clin. Microbiol.* 25:115, 1987.

42 Hill, W.E., Madden, J.M., McCardell, B.A., Shah, D.B., Jagow, J.A., Payne, W. L., and Boutin, B . K. Foodborne enterotoxigenic *Escherichia coli:* detection and enumeration by DNA colony hybridization. *Appl. Environ. Microbiol.* 45:1324, 1983.

43. Echeverria, P., Taylor, D.N., Seriwatana, J., and Moe, C. Comparative study of synthetic oligonucleotide and cloned polynucleotide enterotoxin gene probes to identify enterotoxigenic *Escherichia coli*. *J. Clin. Microbiol.* 25:106,1987.

44. Hill, W.E., Payne, W.L., Zon, G., and Mosely, S.L. Synthetic oligonucleotide probes for detecting heat-stable enterotoxin-producing *Escherichia coli* by DNA colony hybridization. *Appl. Environ. Microbiol.* 50:1187, 1985.

45. Wood, P.K., Morris, Jr., J.G., Small, P.L.C., Sethabutr, 0., Toledo, M.R.E., Trabulsi, L., and Kaper, J.B. Comparison of DNA probes with the Sereny test for identification of invasive *Shigella* and *Escherichia coli* strains. *J. Clin. Microbiol.* 24:498, 1986.

46. Nataro, J.P., Baldini, M.M., Kaper, J.B., Black, R.E., Bravo, N., and Levine, M.M. Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. *J. Infect. Dis.* 152:560, 1985.

47. Levine, M.M., Xu, J., Kaper, J.B., Lior, H., Prado, V., Tall, B., Nataro, J., Karch, H., and Wachsmuth, K. A DNA probe to identify enterohemorrhagic *Escherichia coli* of 0157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. *J. Infect. Dis.* 156:175, 1987.

48. Skirrow, M.B. *Campylobacter* enteritis: a "new" disease. *Brit. Med. J.* 2:9, 1977.

49. Kaijser, B. *Campylobacter jejuni/coli*. *APMIS*. 96:283, 1988.

50. Butzler, J.P. and Skirrow, M.B. *Campylobacter* enteritis. *Clin. Gastroenterol.* 8:737, 1979.

51. Karmali, M.A. and Fleming, P.C. *Campylobacter* enteritis in children. *J. Pediatr.* 94:527, 1979.

52. Blaser, M.J. and Reller, L.B. *Campylobacter* enteritis. *N. Engl. J. of Med.* 305:1444, 1981.

53. Centers for Disease Control. Waterborne *Campylobacter* gastroenteritis -Vermont. *Morbid. Mortal. Weekly Rep.* 27:207, 1978.

54. Mentzing, L.0. Waterborne outbreaks of *Campylobacter* enteritis in central Sweden. *Lancet.* ii: 352, 1981.

55. Palmer, S.R., Gully, P.R., White, J.M.,Pearson, A.D., Suckling, W.G., Jones, D.M., Rawes, J.C.L., and Penner, J.L. Waterborne outbreak of *Campylobacter* gastroenteritis. *Lancet.* ii: 1002, 1978.

56. Knill, M., Suckling, W.G., and Pearson, A.D. Environmental isolation of heat-tolerant *Campylobacter* in the Southampton area. *Lancet.* ii: 1002, 1978.

57. Taylor, D.N., Brown, M., and McDermott, K.T. Waterborne transmission of *Campylobacter* enteritis. *Microb. Ecol.* 8:347, 1982.

58. Blaser, M.J., Berkowitz, I.D., La Force, F.M., Cravens, J., Cravens, L.B., Reller, L.B., and Wang, W.L.L. *Campylobacter* enteritis: clinical and epidemiological features. *Ann. Intern. Med.* 91:179, 1979.

59. Carter, A.M., Pacha, R.E., Clark, G.W., and Williams, E.A. Seasonal occurrence of *Campylobacter* spp. in surface waters and their correlation with standard indicator bacteria. *Appl. Environ. Microbiol.* 53:523, 1987.

60. Oosterom, J., Vereijken, M.J.G.M., and Engels, G.B. *Campylobacter* isolation. *Vet. Q.* 3:104, 1981.

61. Bolton, F.J. and Robetson, L. A selective medium for isolating *Campylobacter jejuni/coli*. *J. Clin. Pathol.* 35:462, 1982.

62. Bolton, F.J., Hinchliffe, P.M., Coates, D., and Robertson, L. A most probable number method for estimating small numbers of *campylobacters* in water. *J. Hyg. Camb.* 89:185, 1982.

63. Mathewson, J.J., Keswick, B.H., and Du Pont, H.L. Evaluation of filters for recovery of *Campylobacter jejuni* from water. *Appl. Environ. Microbiol.* 46:985, 1983.

64. Lauwers, S., De Boeck, M. and Butzler, J.P. *Campylobacter* enteritis in Brussels. *Lancet.* i:604, 1978.

65. Bolton, F.J., Hutchinson, D.N., and Coates, D. Blood-free selective medium for isolation of *Campylobacter jejuni* from feces. *J. Clin. Microbiol.* 19:169, 1984.

66. Kaplan, R.L. *Campylobacter. In:* Lennette, E. (ed.), Manual of Clinical Microbiology, 3rd ed. American Society for Microbiology, Washington, D.C., 1980.

67. Hutchinson, D.N., Bolton, F.J., Jones, D.M., Sutcliffe, E.M., and Abbott, J.D. Application of three typing schemes (Penner, Lior, Preston) to strains of *Campylobacter* spp. isolated from three outbreaks. *Epidem. Inf.* 98:139, 1987.

68. Dilworth, C.R., Lior, H., and Belliveau, M.A. *Campylobacter* enteritis acquired from cattle. *Can. J. Publ. Hlth.* 79:60, 1988.

69. Lior, H., Woodward, D.J., Edgar, J.A., Laroche, I.J., and Gill, P. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. *J. Clin. Microbiol.* 15:761, 1982.

70. Penner, J.L., Hennessy, J.N., and Congi, R.V. Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. *Eur. J. Clin. Microbiol.* 2:378, 1983.

71. Lior, H. New, extended biotyping scheme for *Campylobacter jejuni, Campylobacter coli*, and *Campylobacter laridis*. *J. Clin. Microbiol.* 20: 636, 1984.

72. Bolton, F.J., Holt, A.V., and Hutchinson, D.V. *Campylobacter* biotyping scheme of epidemiological value. *J. Clin. Pathol.* 37:677, 1984.

73. Rosef, O., Kapperud, G., and Skjerve, E. Comparison of media and filtration procedures for qualitative recovery of thermotolerant *Campylobacter* spp. from naturally contaminated surface water. *Int. J. Food Microbiol.* 5:29, 1987.

74. Ribeiro, C.D., Gray, S.J., and Price, T.H. A new medium for isolating *Campylobacter jejuni/coli*. *J. Clin. Pathol.* 35:1036, 1982.

75. Bolton, F.J., Coates, D., Hinchliffe, P.M., and Robertson, L. Comparison of selective media for isolation of *Campylobacter jejuni/coli*. *J. Clin. Pathol.* 36:78, 1983.

76. Goosens, H., De Boeck, M., Coignau, H., Vlaes, L., Van Den Borre, C., and Butzler, J.P. Modified selective medium for isolation of *Campylobacter* spp. from feces: a comparison with Preston medium, a blood-free medium and filtration system. *J. Clin. Microbiol.* 24:840, 1986.

77. Mawer, S.L. *Campylobacters* in man and the environment in Hull and East Yorkshire. *Epidem. Inf.* 101:287, 1988.

78. Mawer, S.L. The pathogenicity of environmental Campylobacters – a human volunteer experiment. *Epidem. Inf.* 101:295, 1988.

79. Dutka, B.J. Coliforms are an inadequate index of water quality. *J. Environ. Health.* 36:39, 1973.

80. Edberg, S.C., Allen, M.J., Smith, D.B., and the National Collaborative Study. National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and *Escherichia coli* from drinking water: comparison with the standard multiple tube fermentation method. *Appl. Environ. Microbiol.* 54:1595, 1988.

81. Evans, T.M., Seidler, R.J., and Le Chevallier, M.W. Impact of verification media and resuscitation on accuracy of the membrane filter total coliform enumeration technique. *Appl. Environ. Microbiol.* 41:1144, 1981.

82. Camper, A.K. and McFeters, G.A. Chlorine injury and the enumeration of waterborne coliform bacteria. *Appl. Environ. Microbiol.* 37:633, 1979.

83. Le Chevallier, M.W., Cameron, S.C., and McFeters, G.A. New medium for improved recovery of coliform bacteria from drinking water. *Appl. Environ. Microbiol.* 45:484, 1983.

84. Le Chevallier, M.W., Cameron, S.C., and McFeters, G.A. Comparison of verification procedures for the membrane filter total coliform technique. *Appl. Environ. Microbiol.* 45:1126, 1983.

85. Geldreich, E.E., Clark, H.F., Huff, C.B., and Best, L.C. Fecal coliform organism medium for the membrane filter technique. *J. Am. Water Works Assoc.* 57:208, 1965.

86. Stuart, D.G., McFeters, G.A., and Schillinger, J.E. Membrane filter technique for the quantification of stressed fecal coliforms in the aquatic environment. *Appl. Environ. Microbiol.* 34:42, 1977.

87. Dufour, A.P., Strickland, E.R.,and Cabelli, V.J. Membrane filter method for enumerating *Escherichia coli. Appl. Environ. Microbiol.* 41:1[illegible]52, 1981.

88. McDade, J.E., Shepard, C.C., Fraser, D.W., Tsai, T.R., Redus, M.A., and Dowdle, W.R. Legionnaires disease: isolation of a bacterium and demonstration of its role in other respiratory diseases. *N. Engl.. J. Med.* 297:1197, 1977.

89. Brenner, D.J., Steigerwalt, A.G., Gorman, G.W., Wilkinson, H.W., Bibb, W.F., Hacket, M., Tyndall, R.L., Campbell, J., Feeley, J.C., Thacker, W.L., Skaliy, P., Martin, W.T., Brake, B.J., Fields, B.S., NcEachern, H.V., and Corcoran, L.K. Ten new species of *Legionella. Int. J. Syst. Bacteriol.* 35:50, 1985.

90. Thacker, W.L., Benson, R.F., Staneck, J.L., Vincent, S.R., Mayberry, W.R., Brenner, D.J., and Wilkinson, H.W. *Legionella cincinnatiensis* sp. nov. isolated from a patient with pneumonia. *J. Clin. Microbiol.* 26:418, 1988.

91. Kirby, B.D., Snyder, K.M., Meyer, R.D., and Finegold, S.M. Legionnaires' disease: clinical features of 24 cases. *Ann. Intern. Med.* 89:297, 1978.

92. Broome, C.V. Epidemiological assessment of methods of transmission of Legionellosis. Zbl. Bakt. Hyg. I. Abt. 0rig. A. 225: 52, 1983.

93. Fliermans, C.B., Cherry, W.B., 0rrison, L.H., Smith, S.J., Tison, D.L., and Pope, D.H. Ecological distribution of *Legionella pneumonhila. Appl. Environ. Microbiol.* 41:9, 1981.

94. Tobin, J.O., Bartlett, C.L.R., Waitkins, S.A., Barrow, G.I., Macrae, A.D., Taylor, A.G., Fallon, R.J., and Lynch, F.R.N. Legionnaires' disease: further evidence to implicate water storage and distribution systems as sources. *Br. J. Med.* 282:573, 1981.

95. Dufour, A.P. and Jakubowski, W. Drinking water and Legionnaires' disease. *Journal AWWA.* 74:631, 1982.

96. Meyer, R.D. *Legionella* infections: a review of five years of research. *Rev. Infect. Dis.* 5:258, 1983.

97. Friedman, S., Spitalny, K., Barbaree, J., Faur, Y., and McKinney, R. Pontiac fever outbreak associated with a cooling tower. *Am. J. Publ. Hlth.* 77:568, 1987.

98. Kaufmann, A.F., McDade, J.E., Patton, C.M., Bennett, J.V., Skaliy, P., Feeley, J.C., Anderson, D.C., Potter, M.E., Newhouse, V.F., Gregg, M.B., and Brachman, P.S. Pontiac fever: isolation of the etiologic agent (*Legionella pneumophila*) and demonstration of its mode of transmission. *Am. J. Epidemiol.* 114:337, 1981.

99. Girod, J.C., Reichman, R.C., Winn, W.C., Jr., Klaucke, D.N., Vogt, R.L., and Dolin, R. Pneumonic and nonpneumonic forms of legionellosis. *Arch. Intern. Med.* 142:545, 1982.

100. Feeley, J.C., Gibson, R.J., Gorman, G.W., Langford, M.C. Rasheed, J.K. Mackel, D.C., and Bain, W.B. Charcoal yeast extract agar: a primary isolation medium for the Legionnaires' disease bacterium. *J. Clin. Microbiol.* 16:437, 1979.

101. Bopp, C.A., Sumner, J.W., Morris, G.K., and Wells, J.G. Isolation of *Legionella* spp. from environmental water samples by low-pH treatment and use of a selective medium. *J. Clin. Microbiol.* 13:714, 1981.

102. Wadowsky, R. and Yee, R.B. Glycine-containing selective medium for isolation of *Legionellaceae* from environmental specimens. *Appl. Environ. Microbiol.* 42:768, 1981.

103. Edelstein, P.H. Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. *J. Clin. Microbiol.* 14:298, 1981.

104. Calderon, R.L. and Dufour, A.P. Media for detection of *Legionella* spp. in environmental water samples. *In:* C. Thronsberry, Balows, A., Feeley, J., and Jakubowski, W. (eds.), *Legionella*, Proceedings of the 2nd International Symposium. American Society for Microbiology, Washington, D.C., 1984.

105. Feeley, J.C. and Gorman, G.W. *Legionella*. *In:* E. Lennette (ed.), Manual of Clinical Microbiology, 3rd ed. American Society for Microbiology, Washington, D.C., 1980.

106. Cherry, W.B. and McKinney, R.M. Direct immunofluorescence tests for diagnosis of legionellosis. *Am. J. Pathol.* 103:448, 1981.

107. Edelstein, P.H. Evaluation of the Gen-Probe DNA probe for the detection of legionellae in culture. *J. Clin. Microbiol.* 23:481, 1986.

108. Pfaller, M.A. Laboratory diagnosis of infections due to *Legionella* species: practical application of DNA probes in the clinical microbiology laboratory. *Lab. Med.* 19:301, 1988.

109. Para, M.F. and Plouffe, J.F. Production of monoclonal antibodies to *Legionella pneumophila* serogroups 1 and 6. *J. Clin. Microbiol.* 18:895, 1983.

110. Joly, J.R. and Winn, W.C. Correlation of subtypes of *Legionella pneumophila* defined by monoclonal antibodies with epidemiological classification of cases and environmental sources. *J. Infect. Dis.* 150:667, 1984.

111. Edelstein, P.H., McKinney, R.M., Meyer, R.D., Edelstein, M.A.C., Krause, C.J., and Finegold, S.M. Immunologic diagnosis of Legionnaires' disease: cross reactions with anaerobic and microaerophilic organisms and infections caused by them. *J. Infect. Dis.* 141:652, 1980.

112. Barbaree, J.M., Gorman, G.W., Martin, W.T., Fields, B.S., and Morrill, W.E. Protocol for sampling environmental sites for legionellae. *Appl. Environ. Microbiol.* 53:1454, 1987.

113. Hussong, D., Colwell, R.R., O'Brien, M., Weiss, E., Pearson, A.D., Weiner, R.M., and Burge, W.D. Viable *Legionella pneumophila* not detectable on agar media. *Biotechnol.* 5:947, 1987.

114. Colbourne, J.S., Dennis, P.J., Trew, R.M., Berry, C. and Vesey, G. *Legionella* and public water supplies *In:* Proc. Int. Conf. on Water and Wastewater Microbiol. 1: 3-1, 1988.

115. Nolte, F.S., Conlin, C.A., Roisin, A.J.M., and Redmond, S.R. Plasmids as epidemiological markers in nosocomial Legionnaires' disease. *J. Infect. Dis.* 149:251, 1984.

116. Collins, C.H., Grange, J.M., and Yates, M.D. A review: mycobacteria in water. *J. Appl. Bacteriol.* 57:193, 1984.

117. Wolinsky, E. Nontuberculosis mycobacteria and associated diseases. *Am. Rev. Respir. Dis.* 119:107, 1979.

118. Kiehn, T.E., Edwards, F.F., Brannon, P., Tsang, A.Y., Maio, M., Gold, J.W.M., Whimby, E., Wong, B., McClatchy, J.K. and Armstrong, D. Infections caused by *Mycobacterium avium* complex in the immunocompromised patients: diagnosis by blood culture and fecal examination, antimicrobial susceptibility tests, and morphological and seroagglutination characteristics. *J. Clin. Microbiol.* 21:168, 1985.

119. Du Moulin, G.C., Sherman, I.H., Hoaglin, D.C., and Stottmeier, K.D. *Mycobacterium avium* complex, an emerging pathogen in Massachusetts. J. *Clin. Microbiol.* 22:9, 1985.

120. Goslee, S. and Wolinski, E. Water as a source of potentially pathogenic mycobacteria. *Am. Rev. Respir. Dis.* 113:287, 1976.

121. Pelletier, P.A., du Moulin, G.C., and Stottmeier, K.D. Mycobacteria in public water supplies: comparative resistance to chlorine. *Microbiol. Sci.* 5:147, 1988.

122. Du Moulin, G.C., Stottmeier, K.D., Pelletier, P.A., Tsang, A.Y. and Hedley-White, J. Concentration of *Mycobacterium avium* by hospital hot water systems. *JAMA.* 260:1599, 1988.

123. Beerworth, W. Culture of mycobacteria from droppings of domestic animals and the role of these bacteria in epizootiology. *Prax. Pneumol.* 25:189, 1967.

124 Bailey, R.K., Wyles, S., Dingley, M., Hesse, F., and Kent, G.W. The isolation of high catalase *Mycobacterium kansasii* from tap water. *Am. Rev. Respir. Dis.* 101:430, 1970.

125. McSwiggan, D.A. and Collins, C.H. The isolation of *M. kansasii* and *M. xenopi* from water systems. *Tubercle.* 55:291, 1974.

126. Du Moulin, G.C. and Stottmeier, K.D. Use of cetylpyridinium chloride in the decontamination of water for culture of mycobacteria. *Appl. Environ. Microbiol.* 36:771, 1978.

127. Petran, E.I. and Vera, H.D. Media for selective isolation of mycobacteria. *Health Lab. Sci.* 8:225, 1971.

128. Lorain, V. and Maddock, S. The effect of anticontamination agents in media for the isolation of mycobacteria. *Dis. Chest.* 50:630, 1966.

129. Mitchison, D.A., Allen, B.W., Carrol, L., Dickinson, J.M. and Aber, V.R. A selective oleic acid albumin agar medium for tubercle bacilli. *J. Med. Microbiol.* 5:165, 1972.

130. Sommers, H.M. and McClatchy, J.K. *Mycobacterium. In:* E. Lennette (ed.), Manual of Clinical Microbiology, 3rd ed. American Society for Microbiology, Washington, D.C., 1980.

131. Tsukamura, M. *In vitro* antimycobacterial activity of a new antibacterial substance DL-8280 – differentiation between some species of mycobacteria and related organisms by the DL-8280 susceptibility test. *Microbiol. Immunol.* 27:1129, 1983.

132. Paramasivan, C.N., Jackett, P.S., Coates, A.R.M., Lowrie, D.B. and Mitchison, D.A. Monoclonal antibodies against *Mycobacterium avium/intracellulare. Indian. J. Med. Res.* 88:13, 1988.

133. Hampson, S.J., Thompson, J., Moss, M.T., Portaels, F., Green, E.P., Herman-Taylor, J., and McFadden, J.J. DNA probes demonstrate a single highly conserved strain of *Mycobacterium avium* infecting AIDS patients. *Lancet.* i:65, 1989.

134. Roberts, M.C., McMillan, C. and Coyle, M.B. Whole chromosomal DNA probes for rapid identification of *Mycobacterium tuberculosis* and *Mycobacerium avium* complex. *J. Clin. Microbiol.* 25:1239, 1987.

135. Butler, W.R. and Kilburn, J.O. Identification of major slowly growing pathogenic mycobacteria and *Mycobacterium gordonae* by high performance liquid chromatography of their mycolic acids. *J. Clin. Microbiol.* 26:50, 1988.

Virological Analysis of Environmental Water Samples

by: Christon J. Hurst, Ph.D
U. S. Environmental Protection Agency
Cincinnati, Ohio 45268
513-569-7331

Introduction

Those viruses which are of greatest concern regarding waterborne disease transmission are the human enteric viruses. These viruses replicate within, and are subsequently shed from, the human enteric tract. Their replication is not necessarily limited to the enteric tract and in many instances an infection can spread to other organs of the body thereby resulting in different recognizable disease syndromes. Table 5.6.1 presents a list of the different groups of human enteric viruses and the illnesses they cause. After being shed in feces, these viruses are transmitted from person to person through the ingestion of fecally contaminated food or water. Enteric viruses are naturally found in municipal sewerage and domestic waste drainage; from there they may enter environmental surface and ground waters. Human recreational activities both within and around bodies of water are another possible source of viral contamination. Once in the water these viruses might survive long enough, for periods of up to several weeks or months depending upon climatic conditions, to complete a cycle of disease transmission back to humans.

Human health concerns about such a waterborne cycle of viral disease transmission have resulted in the development of methods for detecting viruses present in environmental waters and drinking water. Of the human enteric virus groups listed in Table 5.6.1, detection efforts have largely been limited to the adenoviruses, enteroviruses, and rotaviruses. Table 5.6.2 presents a list of the steps that are involved in detecting these and other viruses contained in water samples.

Preparation of Virus Samples

The first two steps in virus monitoring, sample collection, and on-site processing, are often combined into a single operation in which

Table 5.6.1. Human Enteric Viruses

Virus Group	Illness Caused
Adenovirus	Pharyngitis, conjunctivitis, respiratory illness, vomiting, diarrhea
Astrovirus	Vomiting, diarrhea
Calicivirus	Vomiting, diarrhea
Coronavirus	Vomiting, diarrhea
Enterically transmitted non-A, non-B hepatitis virus (hepatitis E)	Hepatitis
Enterovirus (includes polio, echo, coxsackie groups A & B and hepatitis A)	Congenital heart anomalies, herpangina, myocarditis, meningitis, encephalitis, paralysis, rash, fever, hemorrhagic conjunctivitis, respiratory illness, hepatitis
Norwalk virus (possibly a calicivirus)	Epidemic vomiting and diarrhea
Parvovirus	One of two types possibly associated with enteric infection
Reovirus	Fecally shed, but symptoms of illness not well defined
Rotavirus	Vomiting, diarrhea
"Small round viruses" (possibly enteroviruses)	Vomiting, diarrhea

Table 5.6.2. Steps Involved in Virus Monitoring

1. Collection of the water sample.
2. On-site processing of the sample.
3. Preservation and transport of the filter or resulting eluate fluid.
4. Laboratory processing including elution of the filter, if not already done in the field, and secondary concentration of viruses from the resulting eluate fluid.
5. Titration and classification of the viruses.
6. Analysis and assessment of the results.

viruses are concentrated from a water sample through surface adsorption onto the matrix of a cartridge filter. This adsorption occurs during passage of the water sample through an appropriate filter, of which several types are available and these differ in their configuration and chemical composition. While bacteria are large enough that they can be concentrated using filtration methods that rely upon particle size exclusion, common to the "membrane filter" (MF) methods, such techniques are not practical for use in isolating the far smaller enteric viruses from environmental water samples.

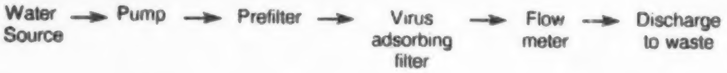
The fact that viruses generally have a net negative electrostatic charge at neutral pH is one of the most important considerations when utilizing adsorption methods for concentrating viruses from water samples. In contrast, filter materials have either a net negative or positive charge, which is established primarily by the filters' chemical composition. If the filter material being used for virus concentration has a net positive charge, then efficient viral adsorption may be achievable without chemically altering the water sample. The adsorption of viruses to positively charged filters is accomplished using a flow-through system of the general design shown in diagram 1 of Table 5.6.3. If the filter material instead has a net negative charge, it is generally necessary to chemically modify the water samples prior to filtration. The most commonly used modification consists of adding a multivalent cationic salt such as aluminum chloride, and a sufficient amount of dilute hydrochloric acid to lower the water sample's pH to 3.5. Required chemical additions can easily be performed using a flow-through system incorporating either a proportioning pump or an in-line injector ahead of the virus adsorbing filter, as shown in diagram 2 of Table 5.6.3. An alternative approach for achieving the required chemical modification consists of batch adjustment of water samples prior to filtration. This latter approach is shown in diagram 3 of Table 5.6.3. If the water sample being examined contains chemical disinfectants such as chlorine, it is necessary to neutralize the residual disinfectant activity prior to filtration of the water sample. Chemicals, such as sodium thiosulfate, can serve to neutralize residual disinfectant activity and are presumed not to have deleterious effect upon the viruses.

The next stage of the virus concentration process consists of recovering adsorbed viruses from the filters. Viral recovery is normally accomplished by a reversal of the adsorption process, achieved during the passage of an eluant fluid, such as beef extract, through the filters. Following its passage through a filter, the eluant solution is termed an eluate, and should contain those viruses which had been adsorbed. This elution process can be performed either in the field, or following transport of the filter to a laboratory. In the latter case, it is necessary that the filter be kept in a sealed sterile container during transport to prevent possible drying and contamination. As a further precaution, the filter should be kept on ice during transport to help protect against thermal inactivation of the adsorbed viruses.

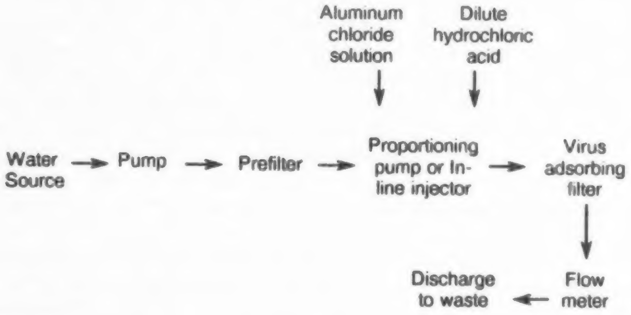
Viruses contained in filter eluates are normally subjected to a secondary concentration procedure prior to their being assayed. Table 5.6.4 presents an outline of the steps used to elute viruses from filters with a beef extract solution, and then to secondarily concentrate them via organic flocculation, a process which involves a low-pH precipitation of proteinaceous compounds that are naturally present in beef extracts. The mechanism by which viruses are concentrated during organic flocculation relies upon their becoming associated with the precipitate that is produced at low pH. This precipitate is collected by centrifugation and then dissolved. The resulting solution is assayed for presence of viruses. Overall, the recovery process concentrates

Table 5.6.3. Equipment Set-up for Achieving Adsorption of Viruses onto Filters

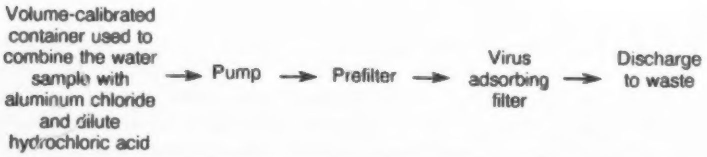
1. Positively charged filters, flow-through system:


Water Source
Pump
Prefilter
Virus adsorbing filter
Flow meter
Discharge to waste

2. Negatively charged filters, flow-through system:


Aluminum chloride solution
Dilute hydrochloric acid
Water Source
Pump
Prefilter
Proportioning pump or In-line injector
Virus adsorbing filter
Flow meter
Discharge to waste

3. Negatively charged filters, batch adjustment and filtration:

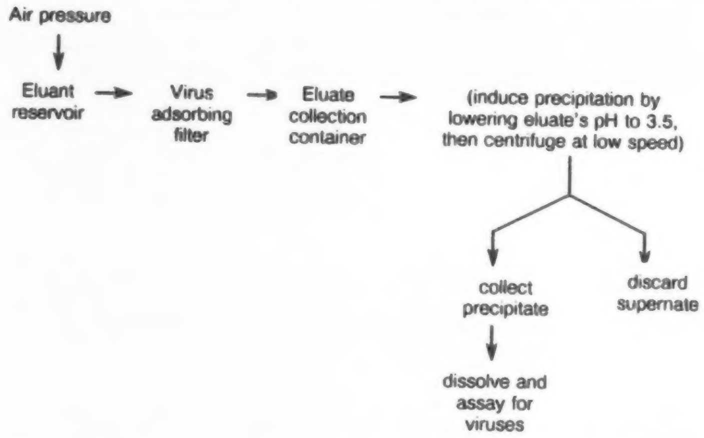

Volume-calibrated container used to combine the water sample with aluminum chloride and dilute hydrochloric acid
Pump
Prefilter
Virus adsorbing filter
Discharge to waste

viruses from volumes of 100 to 400 L of water into approximately 1.5 to 2.0 L of filter eluate, and then secondarily concentrates the filter eluate to between 75 and 100 mL of dissolved low-pH precipitate. This represents net concentration factors of approximately 1,000- to 5,000-fold.

Viral Assay Techniques

The next step in virus monitoring consists of assaying the dissolved low-pH precipitates for presence of infectious enteric viruses. Because these viruses are obligate intracellular parasites, their assay requires the use of live mammalian cells in order to achieve a determination of infectiousness. Mammalian cells can be maintained as cultures in the laboratory, and numerous established cell "lines" are available. A great number of these cell lines are deemed continuous in nature and can be kept in cultivation for a period of years during which time the cells will continue to replicate, allowing the culture to be

Table 5.6.4. Steps in the Elution of Viruses from Filters Using a Beef Extract Solution Plus Subsequent Secondary Concentration of Viruses from the Eluate Fluid


Air pressure
Eluant reservoir
Virus adsorbing filter
Eluate collection container
(induce precipitation by lowering eluate's pH to 3.5, then centrifuge at low speed)
collect precipitate
discard supernate
dissolve and assay for viruses

periodically subdivided. Separate subcultures of the cell lines can then be prepared in commercially available, specially designed tubes, flasks, chambered microscope slides, or petri dishes. Table 5.6.5 contains brief descriptions of four different types of viral assays that have been used for detecting indigenous viruses present in environmental samples. Each of these assay types detects a different viral effect or material that results from the replication of inoculated viruses in cultures of susceptible cells. These assay types differ in the speed with which a successful endpoint can be reached, corresponding to the time required for the virus to produce the particular detected effect or material.

Traditional plaque formation assays require the production of expanding focal areas of cell death, termed "plaques," within cultures of cells. The results of such assays are calculated in terms of plaque forming units per volume of sample. Determination of cell death may be assisted by the incorporation of a vital stain, such as neutral red, into the cell culture medium. This type of assay requires that an infecting virus complete its cycle of replication, killing the initial host cell during the process, and generate progeny viruses which then infect and kill neighboring host cells. Gelling agents, such as agar, are often added to the cell culture media used in plaque formation assays. These gelling agents serve to reduce the rate of viral spread throughout the infected cell cultures, in turn facilitating the detection of more than a single center of infection per culture. While plaque assay techniques are often considered to be traditional for detecting infectious viruses

Table 5.6.5. Comparative Description of Viral Assay Techniques

Type of Assay Technique	Viral Effect or Material which is Detected	Comments
Plaque formation	Focal areas of cell death induced in a culture of cells	Traditional and sensitive but slow. Assay titers may vary depending on factors involved with the assay, including whether improvements like the use of a suspended cell culture technique are employed. Assay results are determined with unaided eye and facilitated by incorporation of a vital stain into the cell culture medium.
Cytopathogenicity	Characteristic alterations in cell morphology resulting from viral infection	This assay technique is older, more rapid, and potentially more sensitive than plaque formation. Results are determined visually by using light microscopy.
Cell Immunofluorescence or Enzyme Immunoassay	Viral proteins produced in cells during the course of infection	Faster than either plaque formation or cytopathogenicity and considered approximately equal in sensitivity. Range of sensitivity is determined by the specificity of antibody preparations used to locate the position of viral proteins within infected cells. Assay results can be determined visually using light microscopy.
In Situ Nucleic Acid Hybridization	Viral nucleic acids produced in cells during the course of infection	Recently developed and either equal in speed or faster than any of the above assay techniques. Range of sensitivity is determined by the specificity of nucleic acid strands used to locate the position of viral materials produced within infected cells. Assay results can be determined visually using light microscopy.

isolated from environmental samples, this type of assay is operationally slow.

Cytopathogenicity assays are based upon the detection of characteristic morphological changes, termed "CPE" as an abbreviation for "cytopathogenic effects," resulting from the replication of viruses in host cells. Such morphological changes often appear prior to the actual death of the host cell; partially for this reason, cytopathogenicity assays generally require less time for completion than do plaque formation assays. The development of cytopathogenicity assays predates that of plaque formation, and cytopathogenicity assays are in some ways more cumbersome. While it is often possible to distinguish several discrete plaques within a single culture of cells, the same individual cell cultures could only be rated as either positive or negative if used for performing viral cytopathogenicity assays. This is due to the fact that viral infection rapidly spreads throughout the entire culture of cells during cytopathogenicity assays. Gelling agents cannot successfully be used to limit viral spread in cytopathogenicity assays because their presence may obscure the development of viral cytopathogenic effects. For these reasons, quantitative cytopathogenicity assays require a greater number of cell cultures than do plaque assays, although for cytopathogenicity assays each individual culture can be smaller in size. Results of cytopathogenicity assays are determined by calculating the most probable number of viral infectious units per volume of sample.

Cell immunofluorescence and cell enzyme immunoassay procedures detect progeny viral proteins produced during the course of viral replication in infected cells. Both of these approaches expose the infected cells to aqueous preparations containing antibodies used as "probes," which specifically recognize and bind to the produced cell-associated viral proteins serving as their "targets." Subsequent visualization of bound antibodies relies upon fluorescing compounds or enzymes that are covalently linked to either the probe antibodies themselves or to "third party" compounds sequentially applied to the infected cells and which adhere to the viral-specific probe antibodies. *In situ* nucleic acid hybridization assays detect progeny viral nucleic acids that are produced in infected cells during the course of virus replication. This approach is operationally similar to those of cell immunofluorescence and enzyme immunoassay, the major difference being that the hybridization techniques utilize nucleic acid strands rather than antibodies as the probes. The nucleotide sequence of these probe nucleic acid strands is complementary to the sequence of the targeted progeny viral nucleic acid material. Following the application of a solution containing the appropriate nucleic acid probe to virally infected cells, the probe and target viral nucleic acids form "hybrid" double stranded nucleic acid molecules. Subsequent visualization of the bound probe nucleic acid strands can be accomplished utilizing either fluorescence- or enzyme-based detection systems similar to those employed for the antibody-based assays.

Both the antibody-based and nucleic acid-based assay techniques require shorter time periods for completion than either the plaque formation or cytopathogenicity assays. This shorter time period is a great advantage in environmental monitoring situations, and results because progeny viral proteins and nucleic acids can be detected early in the viral replicative cycle, often prior to the development of visual cytopathogenic effects or cell death. Results from the antibody or nucleic acid assay techniques can be calculated directly in terms of infectious units per volume of sample by microscopically counting the number of viral positive cells present in the cultures used for the assays. Alternatively, the inoculated cell cultures may be judged positive versus negative and the associated viral titer calculated as a most probable number. The antibody and nucleic acid assay techniques described above are newer to science than are either plaque formation or cytopathogenicity assays. However, the history of using immunofluorescence assays for viral detection spans more than 30 years, and viral applications for *in situ* nucleic acid hybridization span more than a decade.

Assessing the Results of Virological Testing

Enteric viruses can be found both in environmental waters and drinking waters. Viral pollution may be presumed to constitute a potential health hazard for persons consuming or otherwise coming into physical contact with the water. While it is possible to sample waters for the presence of human enteric viruses, practical laboratory techniques are not yet available for determining infectivity of all the viral groups listed in Table 5.6.1. Practical infectivity techniques do exist for members of the adenovirus, enterovirus (including hepatitis A virus), reovirus, and rotavirus groups. The other viral groups listed in Table 5.6.1 can readily be detected by antibody- or nucleic acid-based *in vitro* assays, i.e., assay techniques which do not utilize live host cells. Immune electron microscopy techniques can also be used to detect all of the different virus groups. However, *in vitro* assays and electron microscopy techniques are relatively insensitive when compared with the viral infectivity assays described in this paper and, in their currently available form *in vitro* assays are impractical for use when detecting viruses in environmental water samples. It should also be recognized that, by themselves, the *in vitro* and electron microscopy techniques cannot yield information regarding viral infectiousness. It is hoped that suitable viral infectivity assays will in the future be developed for the other human enteric virus groups listed in Table 5.6.1.

Temporal and Epidemiological Considerations in Virus Sampling

An important factor to consider when assessing the relative merits of examining a water source for the presence of enteric viruses is the likelihood that causative agents might still be in the suspected water source when it is sampled. All viral diseases involve an incubation period between the time a person is exposed to the virus and the subsequent onset of symptoms. The length of incubation for the enteric viruses varies from one virus type to another, ranging from a few days for Norwalk virus to more than one month for human enterovirus 72 (hepatitis A virus). These incubation periods can result in substantial time delays between the exposure of a population to the suspected viral agent and subsequent identification of an ongoing disease epidemic. Another factor to be considered is whether the outbreak pattern suggests that exposure of the population was to acute versus chronic water contamination. Suggestion of chronic contamination involving a virus whose illness has a short incubation period would yield the greatest chance of finding the organism still present in the water source at the time of the outbreak investigation.

Suggested References

1. Akin, E.W. Occurrence of viruses in treated drinking water in the United States. *Water Science and Technology*. 17:689-700, 1985.
2. Belshe, R.B. Textbook of Human Virology. PSG Publishing Company, Littleton Massachusetts, 1984.
3. Berg, G., Safferman, R.S., Dahling, D.R., Berman, D., and Hurst, C.J. U.S. EPA Manual of Methods for Virology. Report No. EPA- 6OO/4-84-O13. U. S. Environmental Protection Agency, Cincinnati, Ohio, 1984.
4. Dahling, D.R. and Wright, B.A. Optimization of the BCM cell line culture and viral assay procedures for monitoring viruses in the environment. *Appl. Env. Microbiol*. 51:709-812, 1986.
5. Hurst, C.J., and Benton, U.N., and R.E. Stetler. Detecting viruses in water. *AWWA*. 81(9) 71-80, 1989.
6. Hurst, C.J. and Stetler, R.E. Recent advances in the detection of human viruses in drinking water. In: Proceedings of the 15th Water Quality Technology Conference. American Water Works Association, Denver, Colorado, 1988. pp. 943-956.
7. Hurst, C.J., McClellan, K.A., and Benton, U.N. Comparison of cytopathogenicity, immunofluorescence, and *in situ* DNA hybridization as methods for the detection of adenoviruses. *Water Research*. 22:1547-1552, 1988.
8. Melnick, J. L., Safferman, R., Rao, V.C., Goyal, S., Berg, G., Dahling, D,R, Wright, B.A., Akin, E., Stetler, R., Sorber, C., Moore, B., Sobsey, M.D., Moore, R., Lewis, A.L., and Wellings, F.M. Round robin investigation of methods for the recovery of poliovirus from drinking water. *Appl. Env. Microbiol*. 47:144-150, 1984.
9. Payment, P., Morin, E., and Trudel, M. Coliphages and enteric viruses in the particulate phase of river water. *Canadian Journal of Microbiology*, 34:907-910, 1988.
10. Payment, P. and Trudel, M. Immunoperoxidase method with human immune serum globulin for broad-spectrum detection of cultivable human enteric viruses: application to enumeration of cultivable viruses in environmental samples. *Appl. Env. Microbiol*. 50:1308-1310, 1985.
11. Payment, P. and Trudel, M. Wound fiberglass depth filters as a less expensive approach for the concentration of viruses from water. *Canadian Journal of Microbiology*. 34:271-272, 1988.

Field Method for Concentrating Viruses from Water Samples

by: Christon J. Hurst, Ph.D.
U. S. Environmental Protection Agency
26 Martin Luther King Drive
Cincinnati, Ohio 45268
(513) 569-7461

Purpose

The procedure described can be used to concentrate suspected human enteric viruses from both environmental freshwaters and tap waters in conjunction with waterborne outbreaks. The produced concentrated sample materials can then be examined for the presence of viruses using other laboratory procedures as desired. The concentration technique described here utilizes a three-stage process consisting of directed virus adsorption onto a solid surface, using in this case a cartridge membrane filter, combined with subsequent use of a proteinaceous eluant fluid to recover the adsorbed viruses and secondary concentration of the eluant fluid. The basic theory behind these methods has been described by Hurst, Benton and Stetler (J. Amer. Water Works Assoc., Vol. 81, No. 9, pp. 71-80, 1989). The volume of water sample that is processed when looking for presence of viruses is not fixed by convention, although a minimum useful quantity would probably be 25 gallons, and an amount of 30 to 50 gallons preferred. For some very low turbidity waters, such as sand-filtered tap waters, the apparatus described here could be used for processing volumes as great as 100 gallons.

Equipment and Reagents

The following materials will be needed and can be prepared ahead of time. Some will be required at the field site where the water sample is to be processed using the filters, others will be used in the laboratory for elution and secondary concentration of the filter eluant. All parts of the equipment and laboratory apparatus that will come into direct contact with either the water sample, beef extract eluant fluid, or "final virus concentrate" must first be thoroughly cleaned and sterilized. All reagents that will be used during any of these operations must also be sterilized. Methods which can be used for sterilization include autoclaving, ethylene-oxide gas treatment, sterile filtration (solutions only!) and chemical treatment using solutions such as calcium hypochlorite or sodium hypochlorite. Sodium hypochlorite solution can be purchased as standard household liquid bleach. Either of these hypochlorite solutions can be used for field sterilization of water storage containers and the other equipment described in this procedure with exception of the filters. The filters described in this study should be sterilized by ethylene oxide gas treatment.

Items to be shipped to the FIELD in preparation for sampling, or otherwise made available there:

(Except for the insulated shipping container and optional stands for supporing the filter holders, which do not need to be sterile, all of the following filtration apparatus components and reagents can be sterilized before they are transported to the field site. The eluant can be sterilized within the 1- gallon polypropylene storage container by autoclaving, the optional sodium thiosulfate solution can be sterilized either by autoclaving or sterile filtration, and all of the equipment except for gasoline powered pumps can generally be sterilized by ethylene oxide treatment. In addition to the equipment listed below, other items that should be available in the field are a compact field type DPD chlorine test kit if you are sampling chlorinated tap waters and therefore need to confirm that you have completely neutralized all free chlorine or other chlorine compounds present in the water sample by adding the prescribed quantity of sodium thiosulfate solution. A portable pH meter would also be needed if you will be sampling waters that need to have their pH adjusted prior to the filtration process. The procedure described relies upon virus-adsorbing filters that are considered electropositive, and as such the only time that pH adjustment would likely be required is when using filters of the 1-MDS type for recovering viruses from waters whose pH is above a value of approximately 7.5. Filters of the Posidyne N-66 type appear to work well at pH levels up to 8.5.

1. Container for storage of water sample prior to concentration

   (If necessary, a new plastic garbage can with fitting cover can be used for this purpose.)

2. Light weight portable electric or gasoline powered water pump fitted for use with either quick-disconnect plumbing adaptors or garden hose couplings. All plumbing adaptors or couplings should either be brass or stainless steel to reduce problems associated with their corrosion during sterilization using chlorine compounds or autoclaving.

   (The style of pumps that you will want to use are commonly referred to as "portapumps" and weigh less than twenty pounds. Larger pumps are not required for this procedure and their use would not offer appreciable advantage since the filtration apparatus has a limited maximum flow rate. If electricity will be available at the site where the sample is to be processed, then take advantage of this fact and plan on using an electric pump of 1/6 to 1/2 hp. Electric pumps are often lighter in weight and easier to handle as compared to gasoline powered pumps. Also, some electric pumps can be sterilized in advance using ethylene oxide gas treatment. The pump chosen for use should be self-priming and, if electric, should be thermally protected. In this case, thermal protection means that the pump will automatically shut off if it overheats. The only parts of a pump which need to be sterile are those which come into contact with the water sample, and these parts of a gasoline powered pump should be sterilized by treatment with hypochlorite solution.)

3. Two filter holders designed for use with standard 10-inch length cartridge water filters, fitted for use with either quick- disconnect plumbing adaptors or garden hose couplings.

   (The filter holders should have clear base sections so that the filtration process can be visually monitored, particularly for air pockets which occasionally develop within the filter holder and impede the water flow. Suitable holders should be available from any of the manufacturers and distributors for the cartridge filters that are listed below. The top of each filter holder should be fitted with a stainless steel finger- operated air pressure release valve connecting to that portion of the filter holder which will be outside the resting position of the cartridge filter. The installation of air pressure release valves in this manner allows for easy elimination of air pockets from the filter holder. Figure 5.7.1 is a diagram of a filter holder equiped with a pressure release valve. These air pressure release valves can be purchased from filtration equipment supply companies and, if necessary, installed by a maintenance shop.)

4. Portable water meter fitted for use with either quick-disconnect plumbing adaptors or garden hose couplings.

   (Light weight water meters with sturdy plastic housings are available from plumbing suppliers.)

5. Four lengths of fiber-reinforced garden hose fitted for use with either quick-disconnect plumbing adaptors or garden hose couplings.

   (Three of the hoses will be needed for connecting the pump, filter holders containing their cartridge filters, and water meter to form the virus concentration apparatus shown in Figure 5.7.2. The fourth piece of hose will be used to direct outflow from the water meter.)

6. One length of strong-walled water supply hose fitted for use with either quick-disconnect plumbing adaptors or garden hose couplings.

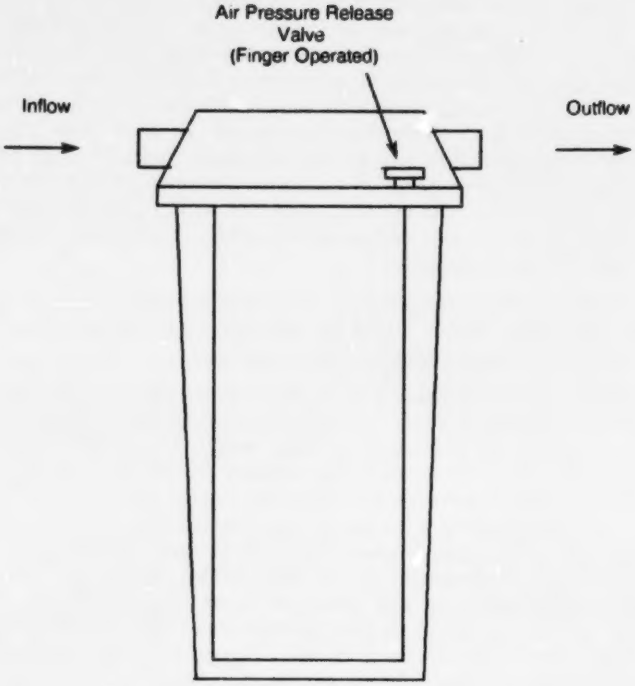


Figure 5.7.1. Filter holder with cartridge filter in place.

(For this hose a coupling will be required on only that end which attaches to the pump. Garden hose would tend to collapse inward during operation of the pump and thus may not be suitable for this particular usage. It may be helpful to have a stainless steel or brass wire strainer fitted to the intake end of the water supply hose when concentrating environmental waters by pumping directly from their source through the filters. Use of a strainer in this instance will help to prevent the water supply hose from clogging.)

7. Two 10-inch cartridge filters.

(For each water sample that is to be processed you will need both a prefilter and a virus-adsorbing filter. These filters should not be reused. The prefilter should be a 3-micron nominal porosity wound polypropylene yarn filter with a hollow perforated stainless steel core, available as stock number M27R10S from Commercial Filters Division, Carborundum Company, Lebanon, Indiana. The virus-adsorbing filter should be either a pleated 0.20 micron porosity charged nylon membrane type, or a pleated 0.45 micron

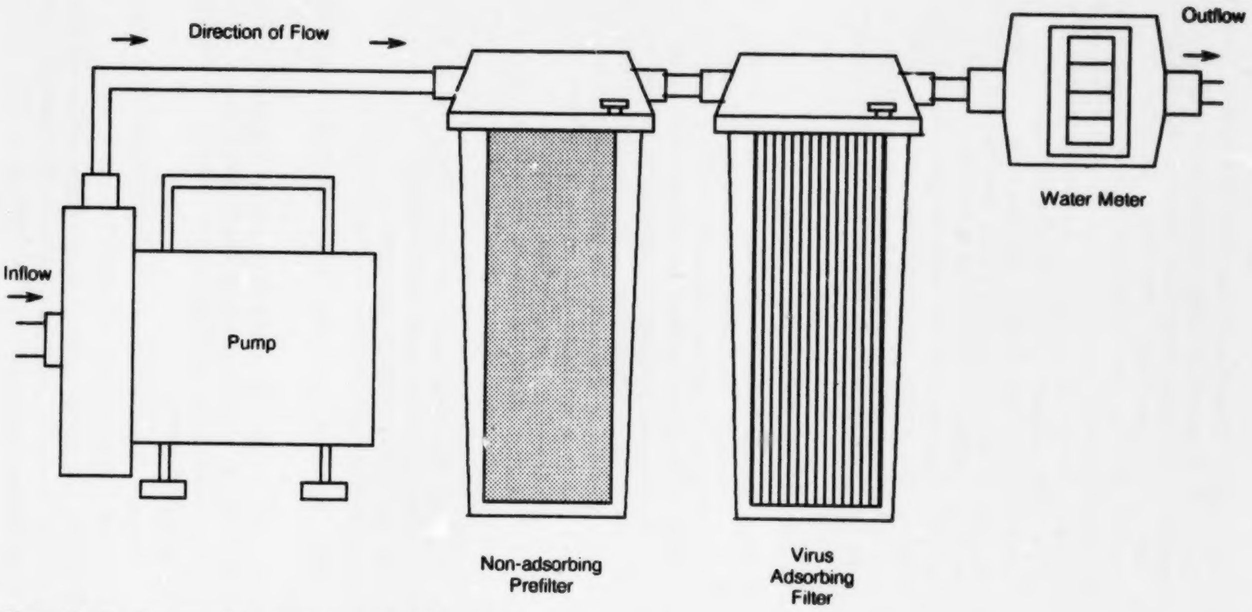

Direction of Flow
Outflow
Water Meter
Inflow
Pump
Non-adsorbing Prefilter
Virus Adsorbing Filter

Figure 5.7.2. Diagram of virus concentration apparatus.

porosity charged glass fiber membrane type. The first of these two is available as a Posidyne N-66 filter cartridge from Pall Trinity Micro Corporation, Cortland, New York, the second is available as a 1-MDS filter cartridge from AMF Cuno Division, Meriden, Connecticut. Both of these virus-adsorbing filters are considered to be positively charged relative to untreated cellulose ester or glass fiber filters, and are therefore suitable for concentrating viruses from water at most ambient pH levels. The charged nylon filter would be preferred for use with water samples of moderately alkaline pH level. If you are instead using the charged glass fiber filter, it would be preferable that dilute hydrochloric acid be used to adjust the pH of the any alkaline water samples to less than 7.5 before filtration. The wound polypropylene cartridge filters and pleated membrane cartridge filters can be presterilized within the filter holders using ethylene oxide gas before they are transported to the field site.)

8. Eluant in plastic storage container.

   (For each water sample to be processed, you will need 1600 mL of sterile, pH 7, 3-percent beef extract solution contained in a separate water-tight, 1-gallon capacity, wide-mouth, screw-capped autoclavable polypropylene container. Polypropylene is preferred for this purpose because it is autoclavable and viruses do not readily adsorb to polypropylene. The eluant solution can be sterilized by autoclaving it inside the polypropylene containers. Containers of this type are available as stock number 2121-0010 from Nalge Company, Division of Sybron Corporation, Rochester, NY. The beef extract solution should consist of 48 grams of microbiological grade beef extract powder dissolved in distilled water. It would be best if at least one half of this powdered beef extract were of the type sold as Beef Extract V by BBL Microbiology Systems, Cockeysville, Maryland. This will help to assure that an easily visible precipitate is produced during subsequent secondary concentration of the filter eluant.)

9. Light-weight insulated container, with ice packs, suitable for mailing.

   (This will be used to ship the used virus-adsorbing filter, inside the polypropylene container of eluant, by overnight delivery service to the laboratory where the elution and secondary concentration steps are to be performed.)

10. Stands to support the filter holders in upright position during the water filtration process. These are optional.

    (Stands of this type can be made of clear plastic or of wood provided that, if made of wood, it is still possible to see the clear base portion of the filter holder while in use. The stands do not need to be sterile.)

11. Solutions of calcium hypochlorite (0.5% w/v) and sodium thiosulfate (50% w/v). These are optional unless you will need to field sterilize either a storage container or other equipment. The thiosulfate solution would also be needed if you are intending to process a sample of previously chlorinated water such as tap water.

(The calcium hypochlorite solution will be used for field sterilization of equipment as necessary and does not, itself, need to be sterilized. Standard household liquid bleach, sold as 5.25% sodium hypochlorite by weight, can be used as a substitute for the calcium hypochlorite solution with the realization that this bleach is appproximately 10-fold stronger than the recommended calcium hypochlorite solution. The sodium thiosulfate solution can be sterilized either by filtration or autoclaving and will be used to neutralize residual chlorine present on field equipment and also to neutralize residual disinfectant activity in samples such as tap water which may already contain free chlorine or chlorine compounds. Pipettes or other means of measuring the hypochlorite and thiosulfate solutions will also be needed.)

Items to be used in the LABORATORY upon return of the virus adsorbing filter and eluate:

1. pH meter, standardization buffers used for calibration of the meter, dilute (1 M) solutions of hydrochloric acid and sodium hydroxide used for pH adjustment, and mechanical stirring apparatus.

   (These items will be used for adjusting the pH of the beef extract eluant and also the dissolved low-pH precipitate, the latter being the end product of the secondary concentration process.)

2. Centrifuge with centrifuge bottles capable of being used at 1350 x g.

   (These items will be used to collect precipitate produced during the secondary concentration process. It is preferable to use a swinging bucket rotor head in the centrifuge. This will deposit the collected precipitate in a layer at the bottom of the centrifuge bottle and thus can simplify dissolution of the precipitate.)

3. Sterile, pH 9.0, 0.15 M solution of dibasic sodium phosphate.

   (This is used to dissolve the low-pH precipitate produced during the secondary concentration procedure.)

4. Sterile filter holder identical to those used in the field for the virus adsorbing filters.

5. Low-capacity electric pump for recirculating pH adjusted eluant through the virus-adsorbing filter contained in a filter holder.

   (A peristaltic pump may be adapted for this purpose. The alternative would be to use a 1-gallon capacity reservoir container or "pressure can", designed for positive pressure filtration of fluids, plus a suitable hose connecting the pressure can to a source of compressed air or nitrogen gas used for pushing the eluant fluid through the filter. Carbon dioxide gas should not be used for this purpose since it may change the pH of the eluant fluid. In common practice only a very limited amount of pressure is required. The amount of pressure applied must never exceed that recommended by the manufacturers of either the filters, filter holders, hoses, couplings, or fluid reservoir. Be mindful that using pressurized gas will necessitate the use of a suitable gas pressure regulator.)

6. Two hoses with fittings that match those on the filter holder to the pump or pressure can.

   (Items 4, 5 and 6 will be used to circulate the eluant fluid through the filter after the eluant has been adjusted to pH 9.5. The use of an antifoaming agent, such as Antifoam C from Dow-Corning Corp., Midland, Michigan, added directly to the eluant may be helpful if foaming is a problem when eluting viruses from the filter. This particularantifoaming compound should be used at a ratio of 0.1 mL antifoam per 100 mL of beef extract eluant solution, for a total amount of 1.6 mL antifoam C in 1600 mL of eluant).

7. Other routine laboratory supplies such as pipettes and beakers will also be needed in addition to a suitable container for storing the final virus concentrate.

Procedure for Concentrating Viruses from Water Samples

The procedure listed below can be used for concentrating viruses directly from unchlorinated surface water or groundwater sources, or else from a stored water sample representing either of these. If the sample being examined instead consists of tap water, then the sample will first need to be collected into a storage container and immediately dechlorinated using sodium thiosulfate stock solution. The latter point is very important since it would be unlikely to find viable viruses in low turbidity water that had been exposed for a prolonged time to even the relatively modest concentrations of chlorine as are normally added to drinking water. If the water sample is to be placed into a storage container for any reason, then that container must first be sterilized. A method for sterilizing water storage containers is described below.

Preparation of water storage containers prior to their use:

The container used for temporary storage of a water sample should be visably clean and sterile, and should not previously have been used for any purpose other than the short term storage of water samples. Large, up to 50-plus gallon capacity, polyethylene containers are available from scientific supply companies and can be used for storage of water samples. In an emergency situation, as mentioned above, a new plastic garbage can may be used for this purpose. Field sterilization of the container should be done by first completely filling it with clear water, and then adding calcium hypochlorite solution (0.5 % w/v) to the water at a rate of 3.8 mL per gallon. A 10% solution of standard household liquid bleach (sold as 5.25 % sodium hypochloride by weight) can be used as a substitute for the calcium hypochlorite solution. The hypochlorite solution should be throughly mixed into the water, and this water then allowed to remain in the storage container for at least 30 minutes. The chlorinated water should then be emptied from the storage container, the container rinsed very thoroughly with clear water, and again completely filled with clear water. Sodium thiosulfate solution should be added to this second filling of water at a rate of 10 mL per gallon. The sodium thiosulfate solution should thoroughly be mixed into the water, and the water then allowed to remain in the storage container for at least 30 minutes. The container can then be emptied and filled with the intended water sample. If the intended water sample consists of tap water or water that for other reasons may have received either chlorine or a chlorinated compound serving as a disinfectant, then the collected water sample should also be dosed with sodium thiosulfate solution at a rate of 10 mL per gallon before it is processed for concentrating any viruses which it may contain. If possible, the water sample should be processed immediately upon its collection using the virus concentration apparatus. If it is necessary to store the water sample, the storage container should be kept covered to prevent contamination and in a cool location to reduce viral inactivation until such time as the water sample can been processed. Prolonged storage of water samples should be done in a refrigerated room.

Field Sterilization of Equipment:

All of the equipment used in the virus concentration apparatus, except for the filters, can be field sterilized if such proves to be necessary. The individual parts should be sterilized separately in order to make certain that all surfaces receive thorough exposure to the hypochlorite disinfectant and that all residual hypochlorite is then neutralized before the water sample is processed through the system. Hoses and assembled filter holders (without filters), with their air pressure release valves open, should be carefully submerged in a solution of clear water containing hypochlorite prepared as described above for treatment of storage containers. Be certain that there are no pockets of trapped air in either the hoses or filter holders, as this may preclude complete sterilization. Keep the hoses and filter holders completely submerged in this chlorinated water for 30 minutes, then remove and drain the hoses and filter holders. Next, similarly treat the hoses and filter holders by submersion for 30 minutes in clear water containing sodium thiosulfate solution prepared as described above for treatment of sample storage containers. The hoses and filter holders should be drained and can then be used immediately or, if to be used at some latter time, their openings should be wrapped with sterile aluminum foil or some other type of sterile covering to prevent contamination.

Field sterilization of pumps can be done by first continuously recirculating clear water containing hypochlorite solution, prepared as described above, through them for 30 minutes. Residual chlorinated water should then be drained from the pump and its necessarily attached hoses. Clear water containing sodium thiosulfate solution, likewise prepared as described above, should then be recirculated through the pump and attached hoses for 30 minutes. Residual water containing thiosulfate solution should then be drained from the pump and attached hoses. The pump and hoses can then immediately be used to process a water sample. If the pump and its attached hoses are not to be used immediately, their openings should receive a sterile covering as described above to prevent contamination.

Concentration of the water sample:

Assemble the virus concentration apparatus as shown in Figure 2, making certain to properly align the pump, filter holders containing their respective filters, and water meter, with respect to their indicated directions of water flow. Be certain that the tops of the filter holders are fully tightened to their bottom sections, that the filter holders contain the correct filters, and that the air pressure release valves on the filter holders are closed. Connect one end of the water supply hose to the pump, and place the other end of this hose into the water being tested, whether it is being taken directly from an environmental water source or from a storage container prepared as described above. Note the reading on the water meter, as water meters usually cannot be reset, and in order to know how much water sample you have filtered it will be necessary to read the meter both before and after the filtration. Turn on the pump and let the sample water pass through the filters and meter. It may be necessary to occasionally release trapped air pockets from the filter holders. The presence of air pockets that form within the filter holders can easily be seen by looking at the clear bottom parts of the holders. Such air pockets should be released as they will otherwise reduce the rate at which water is able to flow through the filters. To release air, first make sure that the holder in question is being held upright so that the trapped air can pass through the release valve. While keeping the holder upright, slightly open the valve until the air has passed through, and then close the valve to keep the water sample from leaking out. After you have filtered the water sample, disassemble the apparatus and allow any remaining water to drain from the filter holders. The filter holders should then be opened carefully, the wound polypropylene pre-filter discarded, and the virus-adsorbing membrane filter aseptically placed into the container of beef extract solution (eluant). The virus adsorbing filter should be kept in the container of eluant and both immediately placed into the insulated container, surrounded with ice packs, and shipped to the laboratory by overnight delivery service.

Laboratory procedure for elution of adsorbed viruses from the filter and secondary concentration of the filter eluant:

Aseptically remove the virus-adsorbing filter from its container of eluant and place the filter into a filter holder that has been presterilized (remember that you must presterilize all reagents and parts of equipment which will come into direct contact with either the water sample, beef extract eluant fluid, or final virus concentrate). The top of the filter holder should be fully tightened to its bottom section and the air pressure release valve closed. The filter holder should then be connected by hoses to either the pump or the pressure can which will hold the eluant. Next, while continuously stirring the filter eluant, adjust it to a pH of 9.5 by addition of sodium hydroxide and hydrochloric acid as necessary. The eluant should then be passed through the filter three times sequentially with the flow of eluant being opposite of that used during the virus adsorption process. It may be necessary to eliminate air pockets from the filter holder using the air pressure release valve. This represents a reversal of the virus adsorption technique described above. The filter can now be discarded. The eluant, including any remaining in the filter holder, pump chamber, pressure can or hoses should then be lowered to pH 3.5 by addition of hydrochloric acid using continuous stirring. During this latter pH adjustment the eluant should become cloudy as an organic precipitate forms. The pH 3.5 eluant should be stirred for 30 minutes, and then centrifuged for 20 minutes at 1350 x g to collect the precipitate. The supernate resulting from this centrifugation should be discarded and the precipitate from all 1600 mL of eluant dissolved in place within the centrifuge bottles using a total volume of 80 mL of sterile, pH 9.0, 0.15 $\underline{M}$ dibasic sodium phosphate solution prepared in distilled water. The pH of the dissolved precipitate, now called "final virus concentrate", should then be checked and if not already between 7.0 and 7.2, adjusted to within that range by adding sodium hydroxide or hydrochloric acid. Antibiotics can be added to the final virus concentrate if such is desired, and the concentrate should then be stored either under refrigeration (for a very few days) or frozen at -70 to -80°C. The final virus concentrate can then be assayed as desired for the presence of viruses. The most commonly used assays for this type of sample are plaque formation, cytopathogenicity (CPE), infected cell immunofluorescence, and in-situ nucleic acid hybridization.

(Note: the filter and its eluant should be processed immediately upon their receipt by the laboratory. If this is not possible, then the filter should be stored refrigerated inside its container of eluant until such time as the elution and secondary concentration processes can be performed. This period of storage should not be longer than 2 or 3 days.)

Analysis of Water Samples for Protozoans

by: Jan L. Sykora
Graduate School of Public Health
University of Pittsburgh
Pittsburgh, PA 15261
(412) 624-3516

Introduction

This study includes two sections. Section I is intended as an introduction to analytical procedures for recovery, isolation, and identification of protozoans from water. Section II focuses on laboratory experiments related to the analytical recovery of *Giardia* cysts from water and sewage.

Most early studies of microbial contaminants in drinking and ambient waters focused on pathogenic bacteria, the exception being the protozoan *Entamoeba histolytica* which causes amoebiasis. This disease was recognized long ago to be waterborne; however, waterborne outbreaks of amoebic dysentery are now rare in the United States and none have been reported in the last few years (1). Recently, the significance of previously unrecognized waterborne illnesses such as primary amoebic meningoencephalitis, giardiasis, and cryptosporidiosis, as well as improvements in analytical techniques, have drawn attention to protozoans.

This chapter focuses on protozoan parasites of major medical significance that are spread by drinking or ambient water. Some of these protozoans are of concern because they are known parasites and/or cause allergic responses, while others may support the growth of disease-causing pathogens (2).

Entamoeba histolytica, free-living pathogenic amoebae, *Giardia lamblia*, *Cryptosporidium* sp., and *Balantidium coli* are the primary parasitic protozoans known to cause a variety of symptoms (3,4,5,6). Some of the ubiquitous protozoans, including nonpathogenic amoebae (*Acanthamoebae*), may cause allergic reactions, such as humidifier fever or hypersensitivity pneumonitis when inhaled (7,8). The role of amoebae and other free-living protozoa in supporting *Legionella* bacteria has been well documented and discussed (9,10,11).

Section I – Analytical Methods

Recovery and isolation of protozoans from water samples utilizes two basic techniques: direct count and cultivation.

The direct count technique is based on microscopic counts of protozoan parasites. It is a complex process that includes filtration, concentration, sample processing by flotation, and microscopic examination. However, highly contaminated samples (such as raw sewage) do not require filtration and some organisms can be counted directly in aliquots concentrated by centrifugation. This technique is used for isolating *Giardia* cysts and *Cryptosporidium* oocysts.

The second isolation technique requires cultivation of protozoans concentrated by membrane filtration on special media (e.g., nonnutrient agar seeded with Gram-negative bacteria). Isolation of free-living amoebae such as *Naegleria* sp. and *Acanthamoeba* sp. is usually accomplished by this method.

Free-Living Amoebae

Several free-living amoebae are pathogenic, including *Naegleria fowleri*, the causative agent of the acute human disease, primary amoebic meningoencephalitis (PAM), a fulminant, usually fatal illness affecting the central nervous system.

N. fowleri is a ubiquitous, free-living amoeba found in diverse freshwater environments such as lakes, ponds, and rivers. Most cases of PAM occur during the summer within 1 week after the victims swim in warm, fresh or brackish waters. The disease is generally associated with diving. From 1965, when this disease was first discovered in Australia, through December 1980, a total of 49 cases have been reported in the United States (12,13).

Pathogenic *N. fowleri* is a thermophilic amoeba, relatively common in warm lakes. This organism has been isolated from lakes and rivers in Florida and manmade reservoirs in Texas (14). Wellings *et al.* have reported that over 50 percent of freshwater lakes in Florida yielded pathogenic *N. fowleri* when their temperatures were 30°C or greater (15).

A new species (*Naegleria australiensis*) has been described and characterized as pathogenic to mice by De Jonckheere (16). However, this amoeba does not grow at temperatures above 45°C and its pathogenicity to mice is lower than that of *N. fowleri*.

In temperate climates the heated effluents from power plants and other industrial facilities discharging cooling waters have been found to provide a perfect environment for maintenance and reproduction of *Naegleria*. In 1974, De Jonckheere and coworkers isolated pathogenic *N. fowleri* from a canal receiving warm effluent from a lead and zinc smelter in Belgium, where a boy swimming in this water contracted PAM in 1973 (16). Additional information on the occurrence of *N.*

fowleri in Florida, Texas, and other parts of the United States was provided by Wellings *et al.* (17), Stevens *et al.* (14), Tyndall *et al.* (18), and Duma (19). Sykora *et al.* detected *N. fowleri* in discharges and/or recipient waters from five out of eight power plants surveyed in western Pennsylvania (5).

In the early 1970s, *Acanthamoeba* was identified as the causative agent of a disease known as granulomatous amoebic encephalitis (GAE). Also at this time, skin and ocular infections (keratitis) caused by *Acanthamoebae* were reported. Due to the current popularity of contact lenses, the incidence of *Acanthamoebic* keratitis has risen. Some free-living amoebae isolated from humidifiers and air conditioning systems have also been reported as likely causative agents of hypersensitivity pneumonitis (8,20).

The role of amoebae in supporting other waterborne pathogens was first documented by Rowbotham, who showed that free-living pathogenic amoebae (*Naegleria* and *Acanthamoebae*) can be infected with *Legionella* organisms (9,21). Tyndall and Dominique established not only that *L. pneumophila* may be used as a food source for free-living amoebae but also that some amoebae cultures become chronically infected and support growth of *Legionellae* (10). A recent report demonstrated that *Acanthamoeba* sp. supported multiplication of *L. pneumophila* in drinking water and that free-living amoebae are frequently present in drinking water in association with *Legionellae* (22). Wadowsky *et al.* demonstrated that hartmanellid amoebae are important determinants of *L. pneumophila* multiplication in some tap water cultures (23).

Detection and Identification of Naegleria fowleri

Cultivation – For each water sample, 250 mL is filtered through a 1.2-µm membrane filter. The exposed filter is cut into halves and placed in an inverted position on nonnutrient agar seeded with *Enterobacter aerogenes* (NNAEa). Bottom sediments including algae, mud, and debris are placed directly into the middle of the same medium. The inoculated plates are sealed, inverted, and incubated at 45°C for up to 8 days. When growth is observed, the amoebae are transferred immediately onto a NNAEa and again incubated for up to 8 days at 45°C to purify the isolate and partially prevent growth of other species of amoebae (Figure 1).

Enterobacter aerogenes **Seed** – Test tubes of nutrient broth are inoculated with *Enterobacter aerogenes* and incubated at 35°C for 24 hours. This broth (0.5 mL) is then pipetted onto trypticase soy agar plates. Plates are allowed to dry and incubate at 35°C for 24 hours. Two mL of sterile water is added to each trypticase soy agar plate, and spread over the agar surface by gently tilting the plate. The bacterial suspension is scraped by a sterile spreader, and pipetted off into a sterile centrifuge tube. The suspension is washed twice by

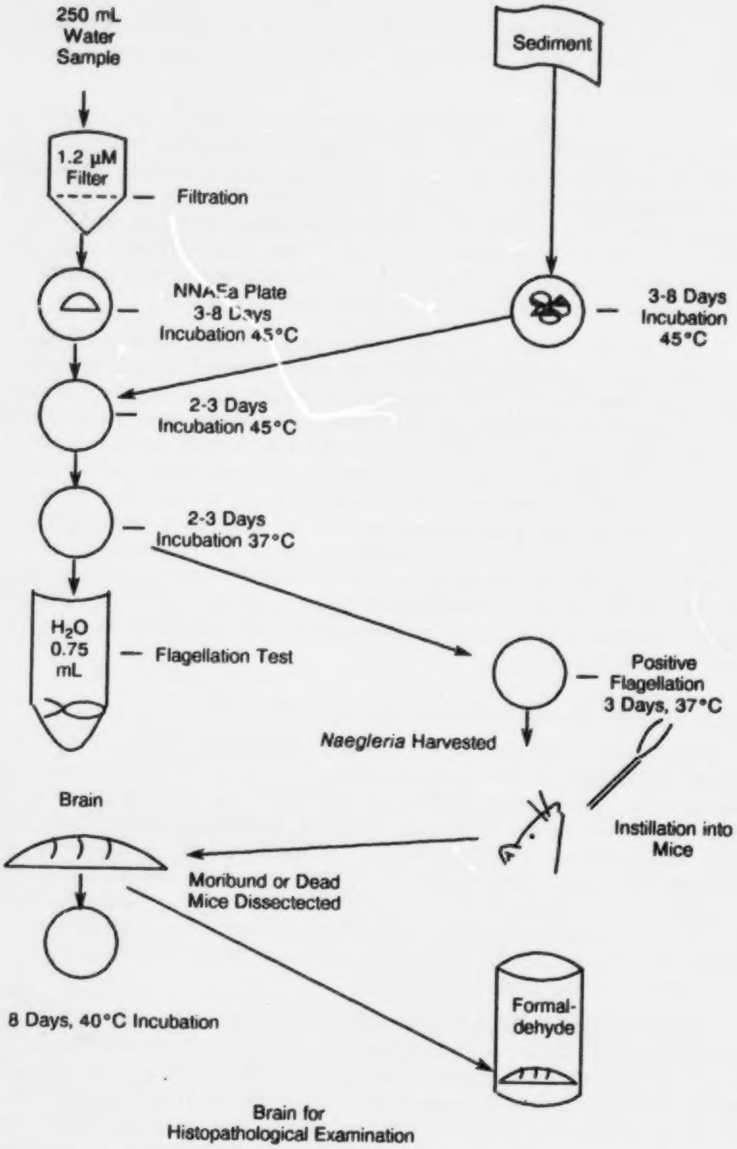

250 mL
Water
Sample
1.2 µM
Filter
Filtration
Sediment
NNAEa Plate
3-8 Days
Incubation 45°C
3-8 Days
Incubation
45°C
2-3 Days
Incubation 45°C
2-3 Days
Incubation 37°C
H2O
0.75
mL
Flagellation Test
Positive
Flagellation
3 Days, 37°C
Naegleria Harvested
Brain
Instillation into
Mice
Moribund or Dead
Mice Dissectected
Formal-
dehyde
8 Days, 40°C Incubation
Brain for
Histopathological Examination

Figure 5.7.1. Isolation and identification of pathogenic *Naegleria fowleri*.

centrifugation for 25 minutes. The resulting bacterial suspension is boiled for 10 to 15 minutes and then cooled. Four drops of the solution are spread onto each nonnutrient agar plate.

Flagellation Test – Usually 3 to 4 days after the start of the second incubation, a small piece of agar with pure amoebic culture is transferred onto a new NNAEa plate and again incubated for 2 to 3 days at 37°C. A piece of agar with the amoebae from this plate is placed in a tube containing 0.75 mL of sterile distilled water and incubated at 37°C. Each tube is examined hourly for 4 consecutive hours using an inverted microscope. Positive amoeba flagellate forming strains are transferred onto a new NNAEa plate and incubated for 3 days at 37°C. These plates are for use in mice pathogenicity tests (Figure 1).

Mouse Pathogenicity Test – A suspension of amoebae, scraped from the plate by a sterile spreader, is prepared in sterile distilled water. The trophozoites are counted using a hemacytometer. Five weanling, white (female) Swiss Webster mice are anesthetized and 30 μL of the amoebic suspension (10^3 to 10^4 of organisms per inoculum) is instilled into their nostrils. Five mice instilled with sterile distilled water are used as controls. The mice are fed and provided with water ad libitum, and kept up to 16 days. Mice with severe neurological symptoms, or those which die, are aseptically dissected. Small sections of the brain are placed in NNAEa and incubated at 40°C to reisolate the pathogenic *Naegleria*. The remainder of the brain can be preserved in buffered formaldehyde, stained, and histopathologically examined. Reisolation of amoeba from the CNS tissue of mice and optional histopathological confirmation are considered to be definitive evidence of the presence of pathogenic *Naegleria* in the tested water or sediment samples.

Detection and Identification of Acanthamoeba

Cultivation – Water samples filtered through 1.2 μm membrane filters or sediment samples are cultivated on 1.5 percent nonnutrient agar containing 2 percent NaCl seeded with *E. aerogenes* (see Detection and Identification of *Naegleria fowleri* for details). All plates are sealed and placed in plastic bags to prevent drying and contamination, and the plates are incubated at 406 for 3 to 8 days. All positive plates are transferred by removing a small piece of agar containing trophozoites and placing it in the center of a new plate (1.5 percent agar without NaCl and incubated at 37°C). Each sample is examined for the presence of *Acanthamoeba* based on its morphological criteria (24, 25).

Pathogenicity Test – Ten young female white Swiss Webster mice are anesthetized with ether and instilled intranasally with known concentrations of amoebae using a Finn-pipette with sterilized plastic tips. Sixty to 90 μL of the suspension are instilled with a known concentration of trophozoites ($10^3$to 10^4 trophozoites/30 μL). A known pathogenic strain of *Acanthamoeba* (e.g., *A. polyphaga*) is instilled into a positive control group consisting of 16 mice (each mouse is instilled with 60 μL with approximately 10^3 to 10^4 trophozoites/30 μL). Sixty μL per mouse of sterile distilled water should be instilled into the control group of four mice. The infected mice are observed for 30 days. The presence of *Acanthamoebae* in dead mice is confirmed by cultivating sections of lungs and brains on NNAEa plates.

Entamoeba histolytica

Entamoeba histolytica has been implicated as a causative agent of human disease since 1875 (26). However, not until 1903 was this amoeba differentiated from previously described *E. coli*, a confusion which created a controversy concerning the pathogenicity of *E. histolytica*. Most investigators now believe that *E. histolytica*, also a potential pathogen, usually is a commensal inhabitant of the intestinal tract (27).

The life cycle of *E. histolytica* is characterized by three stages, i.e., trophozoite, precyst, and cyst, with cyst being the infective stage. The cystic stage is resistant, but it cannot withstand temperatures above 50°C, sunlight, or extended exposure to disinfectants. All three stages of the organism may be found in stool of infected persons.

The pathology of *E. histolytica* is complex and the infection caused by trophozoites inhabiting the lower part of the small intestine may result in 1) a carrier state without symptoms (majority of cases), 2) invasion of the bowel wall without systemic spread, or 3) invasion of the bowel wall followed by systemic spread which could result in lesions in a variety of other organs, most often the liver. Clinical response is variable but most commonly involves acute amoebic dysentery or amoebic diarrhea.

Humans and subhuman primates are the only reservoirs of *E. histolytica*, and the organism is most often transmitted by the fecal-oral route. Sewage contaminated water has been responsible for waterborne outbreaks. The last waterborne outbreak of amoebiasis reported in the United States, which affected 31 individuals, occurred in 1953 (1).

E. histolytica can be identified in environmental samples by direct microscopic count in the sample concentrate or by cultivation. In the past, several *in vitro* cultivation diphasic media have been formulated, including horse serum, ringers solution, and starch (28). A number of investigators developed monobasic media useful for isolation of amoebae from fecal material.

These media usually contain serum, egg products, bacteria, and starch (Balamuth medium) (29). Balamuth medium is now commercially available. Standard Methods recommends the liver infusion medium as modified by Chang and Kabler (30) for *E. histolytica* cultivation (31).

Detection and Identification of E. histolytica

Concentration - Four liters or more of water are filtered through membrane filters (7 to 10 µm pore size), if the concentration of suspended matter is low. Wet filters are flushed with several milliliters of sterile distilled water. The washing could be concentrated by centrifugation. High turbidity water or water suspected of containing a low concentrations of cysts can be processed using high volume filtration described in Detection and Identification of *Giardia* cysts.

Direct Microscopical Examination - Aliquots of concentrated samples are placed in a Palmer-Maloney nanoplankton counting chamber or a Sedgwick-Rafter chamber and are examined under low power magnification for cysts and/or trophozoites.

Cultivation - A concentrated sample is inoculated into a modified liver infusion medium, as proposed by Chang and Kabler (30), incubated for 3 to 6 days at 37°C, and examined microscopically for trophozoites. Concentration of *E. histolytica* in the original water sample could be accomplished using the most probable number technique described in Standard Methods (31).

Giardia

Giardia intestinalis is a common human intestinal parasite and has been the most frequently identified etiological agent of waterborne disease in recent years. The causative agent is a flagellate protozoan characterized by a simple life cycle involving two stages - a cyst and a trophozoite. *Giardia* is shed in the feces of humans and animals, most often in a resistant cyst stage. Each viable, ingested cyst produces two flagellate trophozoites that attach themselves to the epithelial cells of the duodenum and jejunum. The cysts can survive in water for 2 months at 8°C and are more resistant to chlorine than bacteria and *E. histolytica* (32). However, they are highly vulnerable to dessication and temperatures higher than 50°C. The cyst is football shaped, averaging 8 to 12 µm in length by 7 to 10 µm in width. It contains inner structures specific to *Giardia*, called median bodies and an axostyle, which are used for the positive identification of cysts.

At present, since no reliable method exists for culturing *Giardia* cysts from water samples, the only practical technique for *Giardia* detection in water relies on microscopical examination of the sample concentrates. Jakubowski (33), Jakubowski and Ericksen (34), and Vasconcelos (35) reviewed the methods for detecting the *Giardia* cysts in water in great detail and described state-of-the-art sampling and detection of *Giardia* in drinking water. These authors concluded that an ideal technique for the detection of *Giardia* cysts in water would be one that recovers all cysts in a sample simply and inexpensively, enables rapid identification and quantification, and provides information on the infectivity potential of the cyst.

The techniques currently used by most laboratories engaged in detection of *Giardia* cysts in water employ microscopical examination of concentrates derived from several hundred gallons of water. Thus, cyst recovery and positive identification depend on such factors as the amount of water pumped through the filter and the environmental factors affecting the distribution of the cyst in the aquatic environment. More importantly, cyst identification relies on the experience, skill, and persistence of the person analyzing the sample (35). The current sampling and detection techniques for *Giardia* cysts, as described in Standard Methods (31) or as utilized by EPA (36,37) and modified by Schaefer (38), are employed mostly to detect the cyst in drinking water supplies. All methods presently in use involve high volume filtration, cyst concentration, and a microscopical cyst enumeration step. A Lugol's iodine solution is conventionally used to stain the cysts and distinguish them from similar objects such as algal cells. Recently, immunofluorescent techniques have been developed to facilitate cyst identification. These techniques are currently being used by a majority of researchers (39,40,41,37,42) while others still prefer the Lugol's staining procedure (43,44).

The *Giardia* sampling and testing procedure as described in the following section provides general information on techniques which could be modified based on the availability of equipment and the skill of laboratory personnel.

Detection and Identification of Giardia Cysts

Sampling Procedure – In 1976, EPA developed a large volume sampling technique based on virus concentration apparatus then in use (34). Because of the success of this procedure, most investigators now collect samples using *Giardia* sampling equipment consisting of an inlet hose, a plastic filter holder with a 25-cm long, orlon yarn-wound filter (1 μm porosity), an outlet hose, and a water meter. To collect a sample, the inlet hose may be connected to a tap or, if needed, a portable pump can be utilized to pump the water through the cartridge. The volume of water pumped through the filter depends on the purpose of the investigation. A minimum of 380 L (100 gal) is recommended. After sampling is completed, the filter cartridge is placed in a sealed Ziplock® bag containing the water drained from the filter holder, double-bagged, labeled, placed on ice, and processed within 48 hours.

In the laboratory, the filters could be cut in half or divided into fourths by unwinding the yarn into four skeins. The procedure selected depends on the type of filter used. The individual filter sections are rinsed in successive 1-L aliquots of 0.01 percent Tween 20® until the filters appear clean. One percent Tween solution is added as needed to maintain suds. The alternative procedure preferred by some investigators involves backwashing the filter cartridge and using Tween 80® instead of Tween 20®

The sample washed from the filter is concentrated by sedimentation under refrigeration for 24 hours followed by centrifugation or by centrifugation at 600 xg for 3 minutes. One to 2 mL of combined sediment pellets suspended in 70 to 90 mL of 0.01 percent Tween 10® are layered on top of 70 mL of 1.5 M sucrose solution (specific gravity 1.18) in a 250-mL centrifuge bottle. After centrifugation at 800 xg for five minutes the supernatant above the interface and the pellet are discarded. The interface and all underlying sucrose solution are diluted five times with 0.01 percent Tween 20® and further processed by centrifugation at 600 xg for 2 minutes. The resultant sediment is washed twice with Tween 20® in 50-mL centrifuge bottles, transferred into 15-mL centrifuge tubes, concentrated by centrifugation, and stained by Lugol's solution using the Jakubowski and Ericksen procedure (32).

***Giardia* Cyst Identification** – The sediment obtained by this flotation process is examined microscopically using a Palmer-Maloney nanoplankton counting chamber (0.1 mL) and a 45x objective equipped with phase contrast. Positive identification of the *Giardia* cyst is based on dimension, shape, and internal morphological features. For positive identification of a cyst, at least two internal structures must be observed.

Several modifications of this technique involve a membrane filtration procedure proposed by Ongerth (42) and different flotation fluids with a specific gravity of approximately 1.2. In addition to sucrose (Sheather's solution), zinc sulfate and potassium citrate are frequently used by various researchers (31,36,37,40,42,45).

In the early 1980s, a fluorescent antibody technique for identification of *Giardia* cysts in stool samples was developed by Riggs *et al.* (46), who also demonstrated the application of this technique to analyses of large volume water samples (45). Sauch developed an indirect immunofluorescence/phase contrast technique that was successfully used during several giardiasis outbreaks (41).

Initially, immunofluorescent techniques were not widely available because they involved complex procedures for cyst purification and antisera production. However, due to recent commercial availability of the *Giardia* antibodies, this technique is becoming routine.

The indirect immunofluorescent technique described by Sauch utilizes application of anti-Giardia cyst antiserum and fluorescein conjugate to membrane filters through which the concentrated samples are filtered (41). The cysts fluoresce bright green when viewed with ultraviolet light. Rose used direct immunofluorescent techniques consisting of the application of monoclonal antibodies directly conjugated to fluorescein isothiocyanate (37). In both techniques, the filters are contrastained by Evans blue and cleared with glycerol, and the cysts are counted using epifluorescent microscopy.

Cryptosporidium

Giardia and *Cryptosporidium* are enteric parasites that frequently occur in the same environment, and both have high potential for causing waterborne illness. The principal mode of transmission appears to be the fecal-oral route. Both parasites also produce cyst stages that are resistant to standard disinfection.

Cryptosporidium has been classified as a coccidian parasite, but very little is known about its taxonomy and host specificity. In comparison with *Giardia*, it has a relatively complicated life cycle involving sexual and asexual stages. According to Rose (4), two aspects of the taxonomy and life cycle of *Cryptosporidium* increase the possibility of its transmission by water. First, a single species may be responsible for the diarrheal illness in mammals, including humans. Secondly, an environmentally stable oocyst is excreted by infected animals, which may contaminate water.

While the first documented waterborne giardiasis outbreak in the United States occurred in 1965, *Cryptosporidium* received little attention until 1976 when the first human cases were reported and associated with exposure to farm animals (47). Cryptosporidiosis is considered to be a zoonotic disease, transmitted from a variety of domestic and wild animals including calves, deer, and beaver. Prior to 1982, only seven cases of cryptosporidiosis were reported. During that period, most reported infections were in patients with immunodeficiencies other than the acquired immunodeficiency syndrome (AIDS). After that period, however, a significant percentage of AIDS patients developed cryptosporidiosis at some time during their illness. Recent reports show that *Cryptosporidium* is now frequently identified in cases of acute, self-limited diarrheal illness even in immunocompetent hosts in both developed and developing nations. Fecal-oral spread among humans and animals and ingestion of contaminated water seem to be the principal modes of infection.

Recently three Cryptosporidium-caused waterborne outbreaks were reported in the United States. The first, caused by a sewage-contaminated well, resulted in 117 cases (4). The second occurred in winter 1986-1987 in Carrolton, Georgia, when over 10,000 people were affected and 5,000 became ill due to water contaminated with this pathogen (4). In early 1987, another waterborne outbreak was investigated in Arizona. The illness was widespread throughout a community of 27,000 to 36,000 people with the attack rate approximately 71 percent (37,48). The most recent waterborne outbreak of cryptosporidiosis occurred in England in Oxford and

Swindon during February and March 1989. Water treatment plants in these two cities serve approximately 600,000 people.

An investigation of *Cryptosporidium* distribution in environmental samples showed that this parasite is widely distributed in sewage and in surface waters in Arizona, and some cysts were even recovered from water supplies treated by filtration (37,48).

Detection and Identification of Cryptosporidium *Oocyst*

Sample Collection and Processing – The following methodology is based on a technique described by Rose (37). Water samples for *Cryptosporidium* analysis are collected using standard *Giardia* sampling equipment and filtered through 10-inch spun polypropylene cartridge filters (Micro Wynd II, AMF/CUNO Division, Meriden CT) with a nominal porosity of 1 µm. One hundred to 400 gallons of water are filtered at flow rates of 4 to 5 gallons per minute (37). Facilities with pressurized lines can be sampled without the pump by attaching the filter inlet hose directly to a faucet. The filter cartridges containing the sample are double bagged in Ziplock® bags with water from the filter holder, chilled immediately, iced, and processed within 48 hours.

Filter Elution – Filters are processed by backflushing the filter with 2,700 mL of deionized (DI) water eluent containing 0.1 percent Tween 80®. The filters are cut longitudinally, separated from the core, teased apart, and washed three times; each time in one third (900 mL) of the eluent. The washing is performed on an automatic shaker for 10 minutes in a 1-gallon container. The sample is concentrated and combined into a single pellet by centrifugation (1,200 xg for 10 minutes). The final pellet is divided in half and resuspended in 10 percent formalin or 2.5 percent buffered potassium dichromate.

Pellet Processing – Pellets suspended in buffered 2.5 percent potassium dichromate are washed and resuspended with a detergent solution (water with 1 percent Tween 80® and 1 percent sodium dodecyl sulfate) and then homogenized. One drop of antifoam is added to facilitate total sample recovery. Next, the sample is washed and resuspended with the detergent solution, or DI water, and finally sonicated immediately prior to the layering onto a flotation solution.

Centrifugation on Sheather's 500 g sucrose, 320 mL of DI water, and 9.7 mL liquid phenol are used to separate oocysts from sediments. Five mL aliquots of sample are layered onto 10 mL of Sheather's solution. The tubes are centrifuged at 1,200 xg for 10 minutes, and the supernatants are recovered. Additionally, potassium citrate (40 percent solution) could be used to separate oocysts from sediments. The samples suspended in DI water are used for this centrifugation. A 1:3 ratio of sample to media (10 mL to 30 mL) is used. The tubes are centrifuged at 800 xg for 2 minutes and supernatants are recovered.

These recovered supernatants are washed and analyzed using an indirect immunofluorescent antibody technique.

***Cryptosporidium* Oocyst Identification** – The indirect immunofluorescent antibody technique has been modified from previously published immunofluorescent procedures for detection of *Cryptosporidium* oocysts (42,49). Alcohol-cleaned microscope slides containing 10-mm inscribed wells are coated with a 0.01 percent (w/v) solution of poly-L-lysine to improve adherence of *Cryptosporidium* oocysts to the microscope slides (50). Smears from the final resuspended sample pellet are prepared by spreading 0.05-ml portions within the inscribed wells, then air drying and heat fixing them. The entire resuspended sample pellet is processed to maximize sensitivity of the procedure. Smears are covered with 0.05 mL of a murine anti-monoclonal antibody solution, and the slide is incubated in a humidity chamber for 30 minutes at room temperature. Following incubation, slides are washed for 5 minutes in phosphate- buffered saline (pH 7.6). A 0.05-ml portion of fluorescein-conjugated goat anti-murine antibody is added to the wells to detect the presence of bound monoclonal antibodies. The slides are then incubated in a humidity chamber, shielded from light, for 30 minutes at room temperature. Following incubation, they are washed with the phosphate-buffered saline and mounted with buffered glycerol mounting medium. Smears are immediately examined for fluorescent *Cryptosporidium* oocysts, 4 to 6 µm in diameter, using an epifluorescent microscope. The monoclonal and fluorescent conjugate antibody preparations can be obtained commercially from Meridian Diagnostics, Inc. (Cincinnati, Ohio). The working dilutions of the reagents should be those determined by the manufacturer.

The monoclonal antibody supplied by the manufacturer has two advantages over a monoclonal antibody used in a previous study (49). First, the monoclonal antibody preparation supplied by Meridian Diagnostics, Inc., is an IgM antibody and has been found to be more specific than the previously used IgG monoclonal antibody. It is necessary, however, to use the IgM monoclonal antibody in an indirect rather than a direct fluorescent antibody test, since specificity has decreased when attempts have been made to directly conjugate the IgM monoclonal antibody with fluorescein. Secondly, the IgM antibody reacts only with mammalian strains of *Cryptosporidium* and not with avian strains. Since avian strains are not pathogenic to mammals, this additional feature further improves specificity (50).

Section II – Laboratory Studies

It is important to determine the efficiency of recovery from water samples for various organisms. Studies were conducted in our laboratory to determine the efficiency of *Giardia* cyst recovery in distilled water and from sewage samples (51, 52).

For *Giardia* cyst recovery experiments in distilled water, raw sewage and activated sludge, trickling filter, and biodisc effluents, 900 mL samples were each placed in separate glass jars and seeded with equal and known concentrations of *Giardia lamblia* cyst stock. Each jar was mixed thoroughly by inversion and shaking, after which a 250-ml sample was taken from each jar. For Giardia-settling experiments, samples were collected after 2 and 24 hours. These tests were performed at two different temperatures (4° and 21°) in distilled water.

Grab samples of raw sewage and activated sludge, trickling filter, and biodisc effluents were collected from three different sewage treatment plants located in the Pittsburgh region. All samples were taken from a point prior to their entrance into the clarifier. Samples were stored under refrigeration until used and experiments were run within 48 hours of a sample's collection.

In addition, flow composite raw sewage and final effluent samples were collected from a sewage treatment plant also in Pittsburgh. *Giardia* cysts were counted in three samples each consisting of 250 mL of raw sewage, layered on sucrose. Another 250-mL sample of raw sewage was counted without layering on sucrose.

For sample concentration and microscopic examination, samples were centrifuged at 600 xg for 3 minutes. After aspiration of the supernatant, the sediment was stained with two to three drops of Lugol's solution. The sample was then resuspended in 15 mL 0.01 percent Tween 20®, centrifuged again at 600 xg for 3 minutes and aspirated to remove residual Lugol's solution. The remaining sediment was then diluted with 0.01 percent Tween 20® to a concentration appropriate for microscopic examination. Concentrated samples were examined using a hemacytometer (.02 mL) and a light microscope equipped with a 40x objective.

Field samples were also collected from raw water and effluents from the settling tank prior to filtration and postchlorination at the McKeesport, Pennsylvania, water treatment plant. A total of 12 samples were collected in March, April, May, and December of 1987. Samples collected at the water treatment plant were obtained using standard *Giardia* filtration equipment (31). One hundred to 300 gallons of settled water were filtered at a rate of 1 to 2 GPM, while less than 200 gallons of raw water were filtered due to clogging. Following sample collection, filters were refrigerated and maintained at 5°C until analyzed, using the technique by Schaefer and Rice described in the *Giardia* section (36).

Results

Laboratory experiment results show substantial *Giardia* cyst reduction due to layering and flotation procedures. Layering and flotation techniques resulted in 10 to 18 percent of the recovery achieved by direct counts. Additional experiments with two aliquots of the final effluent seeded with a known concentration of cysts (400 cysts/L) showed that 55 percent of the cysts were lost by centrifugation and 92 percent were lost by centrifugation and layering on sucrose (Table 5.7.1).

Table 5.7.1. Effect of Layering for Recovery of *Giardia* Cysts

Procedure	*Giardia* "Seed"/L	Date	Raw Sewage Cysts/L	Raw Sewage Percent Recovery	Final Effluent Cysts/L	Final Effluent Percent Recovery
Layered[a]	None	10/8/87	120	10	4	–
Layered[a]	None	10/8/87	140	12	–	–
Layered[a]	None	10/8/87	220	18	–	–
Not layered[a]	None	10/8/87	1,200	–	–	–
Not layered[a]	400[b]	11/5/87	–	–	180	45
Layered[a]	400[b]	11/5/87	–	–	32	8

[a] Flow composite samples.
[b] 250 mL samples were seeded with 100 *G. lamblia* cysts.

In experiments to investigate cyst recovery from different biological treatment processes, raw sewage and distilled water samples were seeded with *Giardia lamblia* cysts and concentrated using centrifugation without the sucrose layering step. Direct microscopical examination of subsamples revealed relatively high recovery rates ranging from 38 to 72 percent (Table 5.7.2) when compared with a layered sample (8 percent) from Table 5.7.1.

Table 5.7.2. Recovery of *Giardia* Cysts without Sucrose Flotation

Sample	Replicate	Percent Recovery
Distilled water	1	40
	2	63
	3	50
Activated sludge	1	38
	2	47
	3	60
	4	44
Raw sewage	1	72
	2	47
Trickling filter effluent	1	67
	2	55
Biodisc effluent	1	53
	2	56
Activated sludge effluent	1	44

Giardia removal by settling in distilled water at two different temperatures was also investigated. After 2 hours of quiescent settling at room temperature (21°C), only 40 to 50 percent of the cysts were removed from the surface, indicating that settling is not a very efficient means of cyst removal. The removal rate via settling was much lower at the colder temperature, only 0 to 8 percent of cysts were removed from the surface layers after 2 hours of settling at 4°C. After 24 hours of settling, approximately 60 percent of the cysts were removed (Table 5.7.3).

Table 5.7.3. Removal of *Giardia lamblia* Cysts Via Sedimentation in Distilled Water

Temperature (°C)	Settling Time (hr)	Sample Level[a,b]	Percent Removal *Giardia*
21	2	1	40
21	2	2	50
4	2	1	8
4	2	2	0
4	24	1	58
4	24	2	64

[a] Level 1 is 0.5 cm below the surface.
[b] Level 2 is 7.5 cm below the surface.

To study the removal efficiency by sedimentation and flocculation, field samples were obtained from raw and settled water from the McKeesport water treatment plant. Higher concentrations of cysts in settled water were observed in winter months (December and March), indicating that colder water may affect the sedimentation of the cysts, as noted in the laboratory study (Table 5.7.4).

Table 5.7.4. *Giardia* Cysts in Settled and Raw Water (McKeesport)

Date	Settled Water Cyst/100 gal	Raw Water Cyst/100 gal	Percent Cyst Removal	Raw Water Turb, NTU	Settled Water Turb, NTU
3/4/87	18	60	70	18	3.1
3/18/87	9	126	93	48	3.7
4/2/87	0	193	100	38	2.0
4/15/87	7	45	84	15	2.5
5/18/87	0	4	100	37	2.4
12/10/87	10	62	84	-	-

Discussion

The reported laboratory experiments showed that cyst recovery from raw and treated sewage samples is variable and may range from 8 to 45 percent. The results also indicate that recovery is usually quite reproducible when aliquots of one sample are compared (Table 5.7.1). Furthermore, Tables 5.7.1 and 5.7.2 show that more than 50 percent of the cysts could be lost due to concentration by centrifugation and that the layering procedure is responsible for substantial cyst loss. However, results obtained by Sykora *et al.* (53) demonstrated that in spite of the low recovery rate, monitoring of public water supplies with a past history or potential for *Giardia* contamination provides additional information for public water supply protection if *Giardia* cysts are found. A positive result, even without establishing infectivity or viability should stimulate action to modify or provide additional water treatment or water source protection. Such monitoring, though informative, is, however, no substitute for complete and effective treatment of public water supplies. Also, negative results of such monitoring may be misleading due to low recovery of cysts.

Laboratory and field data show that temperature may have a significant effect on the rate of cyst settling. Thus, the higher densities of water in winter may affect the cyst removal efficiency in treatment plants. In addition, the results showing the effect of temperature on cyst settling rate have practical significance. The method for *Giardia* cyst detection published by the American Public Health Association recommends that water samples first be concentrated by centrifugation or by sedimentation under overnight refrigeration (31). As presented in Table 5.7.3, however, after 24 hours of settling under refrigeration (4°C), surface samples contained only approximately 40 percent of the initial concentration of cysts. This indicates that the usefulness of sedimentation under overnight refrigeration as a method of preconcentration should be reevaluated, especially for samples of low turbidity (i.e., drinking water samples). Under these conditions, only centrifugation is recommended for preconcentration of water samples to ensure adequate cyst concentration and better cyst recovery.

References

1. Craun, G.F. 1986. Waterborne Diseases in the United States CRC Press, Inc. Boca Raton, Florida. pp. 1-295.

2. States, S.J. *et al.* 1989. Free-living amoebae and implications for *Legionella*, *Giardia* and *Cryptosporidia*. In: Public Water Supplies. Proceedings AWWA Water Quality Technology Conference, pp. 109-125.

3. Craun, G.F. 1986. Waterborne giardiasis in the United States. *Lancet*. 2:513-514.

4. Rose, J.B. 1988. Occurrence and significance of *Cryptosporidium* in water. *J. AWWA*. 80:(2):53-58.

5. Sykora, J.L. *et al.* 1983. Occurrence and pathogenicity of *Naegleria fowleri* in artificially heated waters. *Appl. Environ. Microbiol.* 45:974-979.

6. Sykora, J.L. *et al.* 1988. *Giardia* cyst in raw and treated sewage. *In:* G.S. Logsdon (ed.), Controlling Waterborne Giardiasis. ASCE, New York.

7. Edwards, J.H. *et al.* 1976. Protozoa as sources of antigens in "humidifier fever." *Nature*. 206:438-439.

8. Sykora, J.L. *et al.* 1982. Amoebae as sources of hypersensitivity pneumonitis. *Environment International*. 8:343-347.

9. Rowbotham, T.J. 1980. Preliminary report on the pathogenicity of *Legionella pneumophila* for freshwater and soil amoebae. *J. Clin. Pathol.* 33:1179-1183.

10. Tyndal, R.L. and E.L.Dominique. 1982. Cocultivation of *Legionella pneumophila* and free-living amoebae. *Appl. Environ. Microbiol.* 44: 954-959.

11. Fields, B.S. *et al.* 1984. Proliferation of *Legionella pneumophila* as an intracellular parasite of the ciliated protozoan *Tetrahymena pyriformis*. *Appl. Environ. Microbiol.* 47:467-471.

12. Martinez, J.A. 1981. Free-living amoebas in human infections. Eastern Pennsylvania Branch of ASM, Philadelphia, PA. p. 57.

13. Martinez, J.A. 1985. Free-Living Amoebas: Natural History, Prevention, Diagnosis, Pathology, and Treatment of Disease. CRC Press, Boca Raton, FL.

14. Stevens, A.R. *et al.* 1977. Isolation of the etiological agent of primary amoebic meningoencephalitis from artificially heated waters. *Appl. Environ. Microbiol.* 34:701-705.

15. Wellings, F.M. *et al.* 1977. Isolation and identification of pathogenic *Naegleria* from Florida lakes. *Appl. Environ. Microbiol.* 34:661-667.

16. De Jonckheere, J.F. 1981. *Naegleria australiensis* sp. nov., another pathogenic *Naegleria* from water. *Protistologica*. 17(3):423-429.

17. Wellings, F.M. *et al.* 1979. Pathogenic *Naegleria:* Distribution in nature. Report No. EPA-600/1-79-018, U.S. EPA, Cincinnati, Ohio.

18. Tyndal, R.L. *et al.* 1978. Isolation of pathogenic *Naegleria* from artificially heated waters. *In:* R.M. Gerhold (ed.), Proceedings of Symposium on Microbiology of Power Plant Thermal Effluents, University of Iowa, Iowa City, pp. 117-123.

19. Duma, R.J. 1981. Study of pathogenic free-living amoebas in freshwater lakes in Virginia. Project Summary. Report No. EPA-800151-80-037. U.S. EPA Cincinnati, Ohio. pp. 1-5.

20. Shapiro, M.A. *et al.* 1983. The role of free-living amoebae occurring in heated effluents as causative agents of human disease. *Wat. Sci. Techn.* 15:135-147.

21. Rowbotham, T.J. 1986. Current views on the relationships between amoebae, *Legionellae*, and Man. *Isr. J. Med. Sci.* 22:678-689.

22. Henke, M., and K. Seidel. 1986. Association between *Legionella pneumophila* and amoeba in water. *Isr. J. of Med. Sci.*, 22:690-695.

23. Wadowsky, R.M. *et al.* 1988. Growth supporting activity for *Legionella pneumophila* in tap water cultures and implication of hartmanellid amoebae as growth factors. *Appl. Environ. Microbiol.* 54(11) 2677-2682.

24. Page, F.C. 1967. Taxonomic criteria for Limax amoebae. *J. Protozool.* 14:499-521.

25. Page, F.C. 1976. An illustrated key to freshwater and soil amoebae. *Freshwater Biological Association.* No. 34.

26. Loesch, F. 1875. Massenhaft Entwickelung von Amoeben in Dickdarm. *Arch. Pathol. Anat. Physiol. Klin. Med.* 65:196-211.

27. Albach, R. A. and Booden T. 1948. Amoebae. *In:* J.P. Kreier (ed.), Parasitic Protozoa, Vol II. Academic Press, New York. pp. 455-506.

28. Dobell, C., and P.P. Laidlow. 1926. On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae. *Parasitology.* 18:283-318.

29. Balamuth, W. 1946. Improved egg yolk infusion for cultivation of *Entamoeba histolytica* and other intestinal protozoa. *Am. J. Clin. Pathol.* 16:380.

30. Chang, S.L. and P.W. Kabler. 1956. Detection of cysts of *Entamoeba histolytica* in tap water by the use of membrane filter. *Amer. J. Hyg.* 64:170.

31. American Public Health Association. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Edition. APHA, Washington, DC.

32. Bingham, A.K. *et al.* 1979. *Giardia* sp: Physical factors of excystation *in vitro* and excystation vs. eosion exclusion as determinants of'viability. *Exp. Parasitol.* 47:284.

33. Jakubowski, W. 1984. Detection of *Giardia* cysts in drinking water. In: S.L. Erlandsen and E.A. Meyer (eds.), *Giardia and giardiasis*, Plenum Press, New York, pp. 263-286.

34. Jakubowski, W. and T.N. Ericksen. 1979. Methods for detection of *Giardia* cysts in water supplies. *In:* Waterborne Transmission of Giardiasis. Report No. EPA-600-9-79-001.

35. Vasconcelos, O. 1984. Methods for testing for *Giardia* in water. Paper presented at Portland *Giardia* conference No. 14-16, 1983. U.S. Environmental Protection Agency Report. pp. 14-73. April.

36 Schaefer, F.W., III, and E.W. Rice. 1982. *Giardia* methodology for water supply analysis. In: Proceedings AWWA Water Quality Technology Conference, Seattle, Washington, pp. 143-147.

37. Rose, J.B.. *et al.* 1987. Detection of *Cryptosporidium* and *Giardia* in environmental waters. *In:* Advances in Water Analysis and Treatment. AWWA, Denver, CO. pp. 417-424.

38. Schaefer, F.W. III. 1984. Personal communication.

39. Riggs, J.L. *et al.* 1988. Recovery, detection, and enumeration of *Giardia* cysts in water. *In:* 6.5. Logsdon (ed.), Controlling Waterborne Giardiasis. ASCE, New York. pp. 13-21.

40. Sauch, J.F. 1984. Purification of *Giardia muris* cysts by velocity sedimentation. *Appl. Environ. Microbiol.* 48(2)454-455.

41. Sauch, J.F. 1985. Use of immunofluorescence and phase-contrast microscopy for detection and identification of *Giardia* cysts in water samples. *Appl. Environ. Microbiol.* 50(6)1434-1438.

42. Ongerth, J.E. *et al.* 1988. Giardia cyst concentrations in river water. *In:* Issues and Answers for Today's Water Quality Professional. AWWA. Baltimore, MD., pp 243-261.

43. Sykora, J.L. *et al.* 1987. Monitoring of water and wastewater for *Giardia*. *In:* Advances in Water Analysis and Treatment, Proceedings AWWA Water Quality Technology Conference, pp. 1043- 1054.

44. Marrocco, F. *et al.* 1987. *Giardia* monitoring and regulation in Pennsylvania surface water supplies. In: Advances in Water Analysis and Treatment, AWWA, Portland, OR. pp 1055-1066.

45. Riggs, J.L. *et al.* 1984. Identifying *Giardia lamblia* by Immunofluorescence. *In:* M. Pirbazari and I.S. Devinney (eds.), Proceedings of the 1984 ASCE Environmental Engineering Specialty Conference, Los Angeles, California, pp. 234-238. June.

46. Riggs, J.L. *et al.* 1983. Detection of *Giardia lamblia* by Immunofluorescence. *Appl. and Environ. Microbiol.* 45(2) 698-700.

47. Janoff, E.N. and L.B. Reller. 1987. *Cryptosporidium* species, a protoan protozoan. *Journal of Clinical Microbiology*. 25(6) 967-975.

48. Rose, J.B.. *et al*. 1988. Method for the recovery of *Giardia* and *Cryptosporidium* from environmental waters and their comparative occurrence. *In:* R.M. Wallis and B.R. Hammond (eds.), Advances in *Giardia* Research. University of Calgary Press, Calgary, Canada, pp. 205-209.

49. Musial, C.E. *et al*. 1987. Detection of *Cryptosporidium* in water using polypropylene cartridge filters. *Appl. Envir. Microbiol.* 53:687.

50. Sterling, C.R. 1987. Personal communication.

51. Sykora, J.L. *et al*. 1989. Monitoring of environmental samples for *Giardia* cysts. In: Proceedings of Fourth Conference on Progress in Chemical Disinfection, April 11-13, 1988, SUNY, Binghamton, New York, pp. 61-74.

52. Kim, R.P. and Sykora, J.L. 1989. Removal of *Giardia lamblia* cysts by coagulation and sedimentation processes. *In:* Proceedings of Fourth Conference on Progress in Chemical Disinfection, April 11-13, 1988, SUNY, Binghamton, New York, pp. 75-98.

53. Sykora, J.L. *et al*. 1987. Monitoring as a tool in waterborne giardiasis prevention. *In:* P.M. Wallis and B.R. Hammond (eds.), Advances in *Giardia* Research. University of Calgary Press, Calgary, Canada. pp. 103-106.

Epilogue

The emphasis of this volume is on the prevention of infectious waterborne diseases and the improvement of surveillance, reporting, epidemiologic investigations, and laboratory analyses. In these areas, we cannot rest on past accomplishments. Continuing microbiological challenges are a reminder that our task is yet unfinished. Although the focus of this volume has been on drinking water systems, enteric infections have also been associated with swimming and other recreational activities where water is accidentally ingested. *Pseudomas* folliculitis, pharyngitis, external otitis, and schistosomal dermatitis have been associated with recreational activities involving water contact in hot tubs, whirlpools, and swimming pools or lakes. There is evidence that inhalation exposure is important as well; Pontiac fever caused by *Legionella* has been associated with use of whirlpools. Organisms such as *Legionella* and nontuberculous *Mycobacteria* can contaminate drinking water systems, find conditions favorable for growth, and may be aerosolized into the indoor air through showering and other household uses of water. *Legionella* has also been disseminated in the air environment through cooling towers.

Not only is more work required on infectious waterborne diseases but also on the noninfectious diseases associated with chronic waterborne exposures. In addition to inorganic constituents and radionuclides, more and more organic contaminants are being identified in our drinking water. These organic compounds are contaminants from our industrial society but, as previously noted, can be formed during the disinfection of drinking water. The routes of exposure can be oral, dermal, or inhalant; for some volatile organics, inhalation can be an important route of exposure. The offgassing of radon from some ground-water supplies into the home environment can be as significant a source of exposure in addition to radon entering household air from the decay of radium in underlying bedrock or soils.

Although toxicological data can provide risk estimates for exposures to chemicals and radioactivity, uncertainties still exist – uncertainties which can be minimized only when data are available from human populations. Epidemiology, which is required for the investigation of outbreaks, is also used for establishing associations and determining the magnitude of risk from these chronic exposures. Case-comparison and cohort studies must be carefully designed, conducted, and interpreted if they are to contribute to our understanding of the etiologies of noninfectious disease. Epidemiologic methods have been developed specifically to study chronic exposures and diseases of long latency periods. While certain statistical methods cannot be strictly applied to the study of infectious diseases, these epidemiologic concepts must be considered and used appropriately to help avoid bias and improve inferences from outbreak investigations.

G.F.C.

*U.S. GOVERNMENT PRINTING OFFICE: 1990--726-121/D09658